

Why honest money is important

Scandals in the financial markets in Britain and the United States point to the need for new rules and regulations, but also for different fiscal policies.

ONE common view of the scandals that have preoccupied the financial communities in New York and London since last May is that nothing much better can be expected of those concerned and that the games they play are unreal, as if with the notional money provided with the popular board games people give each other at Christmas time. But nothing could be further from the truth. First, to the extent that the huge sums of money that slosh around in the financial markets (and sometimes spill over into unauthorized pockets) are increasingly the funds invested by insurance companies, pension funds and other financial institutions whose liquidity derives from people at large, it is our money that they are playing with (and sometimes stealing). Second, because the world's stock markets can have a decisive influence on the well-being and even survival of particular businesses, well-established and still nascent, their conduct determines the use made of science through technology. Third, because the efficiency and stability of the financial markets help to underpin other financial institutions such as banks and even governments, their misconduct is potentially a threat to the economic system as a whole.

The latest development, affecting the London-based Irish company Guinness, is as good an illustration of the dangers as any. For years it has been a marvel that the company should have been able to make its way in the world successfully by making and selling an opaque alcoholic beverage which is unattractively coloured black (with white froth). Four years ago, Guinness set out to diversify and, in the process, to grow, at which nobody can complain. Two years ago, it bought a substantial whisky distiller called Arthur Bell. Last year, it embarked, successfully in the event, on buying what amounts to virtually the remainder of the Scottish whisky industry, the assets of the Distillers Company. The bid was hotly contested by another British group. Guinness paid for most of the purchase by exchanging its own shares for those held by shareholders of Distillers. Now it has emerged that the directors of Guinness (four of whom have been sacked in the past few days) engaged in a variety of schemes for rigging the price of Guinness shares on the London stock market in the crucial days before the competition for Distillers closed.

Revelations

For months ahead, prurient interests will be sated by revelations still to come, but already it is clear that many of the schemes used by Guinness to prop up the price of its shares last year were in contravention of British company law, which forbids the purchase by a company of its own shares except with the consent of the shareholders as a whole. This sensible and equitable provision is the reason why British companies cannot buy off unwelcome shareholders as in the US practice called 'green-mail', a term derived from blackmail by means of a reference to the colour of a dollar bill. Guinness appears to have sought a way round this provision by recruiting others to buy on its behalf, offering to indemnify them against loss (which is also illegal). In the process it has emerged that Guinness invested \$100 million in a fund run by the disgraced Wall Street operator Ivan F. Boesky (see *Nature* 324, 289; 1986), presumably as a *quid pro*

quo for something, that a London and a Swiss bank hold Guinness shares together with interest-free deposits of Guinness money and that the directors now in charge of the company have so far been unable to discover why sums amounting to £27 million were paid to persons not yet identified for assistance with the bid for Distillers.

Embarrassment

How far the dust from this scandal will settle is anybody's guess. Even though the British government took the initiative in bringing Guinness to book by appointing two inspectors armed with new powers to take evidence under oath, it is now embarrassed for two reasons: its espoused cause of the shareowning democracy is made to look a little like tinsel, while its belief that financial institutions should be encouraged to regulate themselves by voluntary agreement is now made to seem even more hollow than during the parliamentary debates on the Financial Services bill last year. The curious anomaly that the body known as the Takeover Panel, the source for example of the rule that companies bidding for others should not persuade third parties secretly to act in concert with them, has no statutory authority, will clearly have to be removed. The government's embarrassment can only be increased if, as seems quite possible, it emerges that Guinness loses not merely the sums it spent on supporting its own shares, the cost of the lawsuits yet to come and even of the fines that could be levied if the company as distinct from its errant directors are taken to court, but its independence as a company. Yet those who will lose financially will mostly be ordinary people, those who work in laboratories included, whose pensions will be a little lower than they might have been, and whose insurance premiums will cost a little more.

Self-interest

The aggregate of individual self-interest is thus a sufficient reason why the financial markets should be well regulated, but there are also economic and industrial reasons why regulation should be effective. Since the invention of the concept of the joint-stock company in seventeenth-century England, corporations have been an essential means of assembling substantial amounts of capital in the pursuit of industrial and commercial objectives of all kinds, and have thus been the means why which an efficient division of labour could be sought. To that end and also for the sake of equitability among stockholders, it is also essential that shares should be sold and bought, whence the necessity of stock exchanges. The buying and selling of technically intricate financial instruments, such as rights to buy or sell stock at some point in the future, are similarly means by which the markets are made more efficient. The striking development of the recent past has been the internationalization of this process, which promises a more rational division of labour than could have been dreamed of in Adam Smith's time. The corruption of the markets by dishonesty, both by siphoning off undeserved gains and by engendering distrust of them, works in the other direction.

Unfortunately, it is easier to state this platitude than to prescribe how governments should respond. Part of the difficulty is



defining what constitutes dishonesty. Dealing with the international character of the problems now arising will be difficult; regulatory authorities in the United States, Western Europe and Japan have recently been consulting about some aspects of their work, but they appear to have in mind more an exchange of information on blatant wrong-doing than the development of uniform sets of rules that will be made uniformly applicable. Should not the Organisation for Economic Cooperation and Development take the initiative in at least defining what needs to be done, by central banks and governments? The need must not be left to the inclinations of self-satisfied regulators.

Problems

Even domestically, governments (other than that of Japan) have problems enough to grapple with. Both in the United States and Britain, for example, there are problems about takeovers. In Britain, the Guinness scandal has given a new lease of life to the body of opinion that takeovers are in some sense morally reprehensible, but that is mistaken. Everywhere, the managements of badly run companies seek to avoid purchase even when they must know that it is in the best interests of everybody but themselves. Even when the objective is to acquire a company only then to break it into pieces, a takeover may make economic sense. It is not even reprehensible that, in the United States, the most spectacular takeovers of the past few years have been financed by what are called 'junk bonds' (which are promises to pay high interest rates without the backing of collateral); if people willingly shoulder the risk, nobody can complain. The difficulty, typified by an attempt mounted in the past two months to buy up the British company Pilkington (which invented the float glass process) is that a company's best defence against being bought up may be to base its strategy on a timescale no longer than that of the people who play the markets for short-term gain. Nobody could begin to guess how many companies in Britain and the United States have abandoned sound long-term research projects for the sake of making their annual accounts read well enough to keep marauders at bay. What governments and corporations together need to find is a mechanism for sustaining technical excellence within the maelstrom of the markets have become.

Would all this not be simpler if people were less concerned with short-term gains, from the markets or from high interest rates? That nostalgic cry deserves attention in only one sense, but an important one affecting chiefly the United States, now the largest debtor nation on the international scene which has also accumulated a formidable amount of domestic debt, chiefly in states affected by the recent slump in the price of oil and agricultural products, but with junk bonds accounting for more than \$125,000 million as well. While Congress and the administration shape up for a bruising six months of argument about the budget for next year, nobody seems to bother about the sense of financial unreality induced by these huge amounts of debt. Indeed, the unreality has now spread to the US government itself, which seems wedded to creative accounting that would be reprehensible, and perhaps even illegal, if practised by public corporations. The *Wall Street Journal* reported last week that the Farm Credit system, to which many US farmers are indirectly in hock to the tune of more than \$70,000 million, will in future keep two sets of books, only one of which will record the proportion of the system's assets (loans) that have gone sour, and which have thus become federal obligations. The same practice, it seems, is spreading to other off-budget agencies of the US government, which are enabling unprofitable banks (and even bankrupt countries) to remain in business rather than stomach the embarrassment of recognizing that their assets have turned into obligations. When governments behave like that, how can they impose higher standards on the public companies they regulate? Or if, like Guinness, their hope that things will come right by accident is disappointed, how can they promise us that the system will not collapse like a house of cards? □

UNESCO in transition

The next director-general of UNESCO should be a scientist of some distinction.

BRITAIN, the United States and Singapore, which have walked out of UNESCO in the past two years, will have no direct say in the appointment of a successor to Dr Amadou Mahtar M'Bow, the director-general who retires at the end of the year, but they will have as vivid an interest in the outcome of the next four months of diplomacy, which will determine whether they are eventually tempted back into membership. Against the odds, they will be surprised to learn, at least two serious candidates have entered the field, together with a number of others put forward by member governments much in the spirit in which state delegations to political conventions in the United States propose favourite sons for an important nomination only to bargain away their later withdrawal. Why should sensible people seek such a thankless job?

Idealism, it seems, occasionally triumphs over cynicism, and optimism over disappointment. While the past few years of UNESCO's history have been marred by political squabbling and administrative confusion, the reasons why the organization was originally founded (in 1947) are probably more valid now than then. International problems touching education, science and culture persist, as do opportunities in these same fields. Moreover, UNESCO's record over forty years, while not nearly as distinguished as its founders hoped, has not been all dust and ashes. There is, after all, an international copyright convention (which is urgently in need of extension to cover intellectual property in general). In science, UNESCO has functioned chiefly as a sponsor of good works, but some of these have been very good. The international community has benefited greatly from UNESCO's early sponsorship of the International Council of Scientific Union and, latterly, from its support of the International Centre of Theoretical Physics at Trieste. UNESCO has gone off the rails by trying to be what other UN agencies mostly are — an aid agency for the developing countries of the world encumbered by a uniquely impoverished bureaucracy.

UNESCO's small harvest of success is a pointer to the choice of a new director-general. Even without the contributions of the United States and the others who have allowed their contributions to lapse, UNESCO is a substantial organization. Success requires that it should concentrate on the tasks it has discovered to be within its competence, which are mostly scientific in their connections. So logic would suggest that the next director-general should be a distinguished scientist with some experience of managing international organizations. The ideal would be that he or she should be from a developing country and thus well placed to disarm the complaint of UNESCO's largest constituency that it has been hijacked by the rich countries of the world. The reference books will show that one of the few people who might fit this demanding prospectus is Professor Abdus Salam, who shared with Weinberg and Glashow the Nobel Prize for the electro-weak theory, and who is now (among many other things) director of the Trieste institute. But would such an active physicist stand for such an office?

The surprise, apparently, is that Salam is even challenged by the idea, which appears also to appeal to several of the member governments of UNESCO. There is some evidence that his backers may be found to include the Soviet Union. Disappointingly, however, Pakistan (where Salam was born) seems bent on nominating its foreign minister, General Ayyub Khan. Would it not make sense that India, which has not declared itself, should make a gesture towards the resolution of the endless problems of the subcontinent by taking up the case for Salam? And that the United States and Britain, knowing that they will be lobbied to renew their membership once the issue of the director-general is settled, should use their influence in advance to ensure that UNESCO is competently led again?

Row in United States over schools AIDS education

- Controversial plans to include children
- Education and Health departments feud

Washington

A row has broken out in the White House over the proposed plan of the US Public Health Service (PHS) to prevent and control the spread of AIDS (acquired immune deficiency syndrome) by a radical education programme that could include children under the age of eight.

The row coincides with the visit to the United States of Britain's health minister, Norman Fowler, who last week was similarly embroiled in criticism as he launched the United Kingdom's education campaign aimed at 18-year-olds and above.

The battle in the United States broke out last week at a meeting of the cabinet-level Domestic Policy Council. A representative of Education Secretary William Bennett criticized PHS for pursuing a "morally empty" approach to an educational programme on AIDS; at least two cabinet members are said to have expressed

say that children should not have sex, and that adult sexual behaviour should be based on "fidelity, commitment and maturity". The programme should also distinguish between heterosexual and homosexual sex, emphasizing that heterosexual sex within marriage is "what most Americans... consider the proper focus of human sexuality." PHS's suggestion that condoms will promote "safe sex" is also questioned. Klenk suggested the report could warn, "If your partner is infected, avoid sex with him or her like the plague".

Congress will also give PHS trouble. Although the administration has requested \$534 million for AIDS research and prevention in its 1988 budget, the administration plan to reduce by 700 the number of new competitive research grants available this year from the National Institutes of Health drew fire. Senator Edward Kennedy (Democrat, Massachusetts) accused PHS of "robbing Peter to pay Paul" with its plan to take \$334 million from the 1987 budget and shift it to 1988. David Baltimore, co-chairman of the National Academy of Sciences committee on a national strategy for AIDS, testified that taking money from other biomedical research to fund AIDS research is a mistake. Sheldon Wolff, the other co-chairman, argued that an effective education and public health campaign would cost \$1,000 million by 1991.

CDC, coordinating all PHS AIDS education activities, will make grants to state and local health authorities totalling \$30 million this year for demonstration, counselling and education programmes. A further \$11.2 million will go for developing health education curricula and providing grants for schools with AIDS education programmes. CDC also has a \$6 million budget for public information campaigns, including an AIDS hotline, information clearing-house and a possible \$1 million advertising campaign.

But Wolff argues that an effective campaign may cost more like \$50-60 million, the amount typically spent to launch a new laundry detergent.

Joseph Palca

• Azidothymidine, a drug that prolongs short-term survival of certain patients with AIDS, last week received a vote of confidence from an advisory panel to the Food and Drug Administration (FDA). The panel recommended that FDA approve the drug for commercial licence as a treatment for AIDS patients with certain opportunistic infections. □

Space shuttle setback

THE efforts of the National Aeronautics and Space Administration (NASA) and Morton Thiokol Inc. to redesign the space shuttle's solid rocket booster, which was the cause of the *Challenger* accident, are not going smoothly. A technical assessment conducted by a special panel of the National Research Council warns that unexpected results from the testing programme may delay returning the shuttle to service, because the planned February 1988 launch makes no allowances for undiscovered problems. The main problem so far is that a new "O"-ring seal, made of a different material supposed to have better low-temperature characteristics, is harmed by rust inhibitor used in the booster. NASA has therefore switched back to the original material, with heaters to ensure the seals do not get too cold. The panel is "concerned" about that development and various other changes that might introduce new critical failure points. □

Jeantet prizes awarded

SYDNEY Brenner, who was made a Companion of Honour in the British New Year's Honours list, is now also £340,000 better off as a result of his share in the 1987 awards of the Louis Jeantet Foundation for Medicine. Of this sum, £40,000 is a personal award and the rest is to be used to create research fellowships within the Medical Research Council's Molecular Genetics Unit. Sharing the prize are Professor Walter Gehring of the University of Basel's Biozentrum and Professor Dominique Stehelin of the Pasteur Institute in Lille, both of whom will use their £300,000 awards to initiate new research groups. □

New austerity in Cuba

CUBA's new austerity programme, aimed at cutting hard-currency imports, includes putting the country on a new, "healthier" diet. Measures announced by Fidel Castro include the elimination of afternoon snacks in all government offices, reduction of milk quotas for secondary school children and for workers' canteen meals, and cuts in sugar quotas to the food industry and in the sugar content of foods. □

Nuclear scientists lose out

ABOUT 170 British nuclear scientists must be content to be the poor relations of a European research project and be paid almost half of the incomes commanded by their colleagues from other community countries working on the same project. The British scientists failed to secure the backing of the European Court of Justice last week. The scientists, paid less than 50 per cent of the salaries of their Community colleagues by the United Kingdom Atomic Energy Authority, work on the Joint European Torus (JET) project at Culham in Oxfordshire and wanted salary parity. □



Bennett accuses PHS of "moral emptiness".

sed outrage at the possibility of their 8- or 9-year-old child learning about anal intercourse at school.

When and how schoolchildren should be informed about AIDS has been a particularly contentious issue. The Centers for Disease Control (CDC) in Atlanta, one of PHS's agencies, are preparing school curricula for AIDS education, and the Surgeon General has urged that education should begin as early as possible. But according to an Education Department source, cabinet members are concerned that PHS has not involved them.

A memorandum dated 13 January from Jack Klenk of the Department of Education, to Gary Noble, PHS AIDS coordinator, details the Education Department's concern about his agency's plans. The memorandum urges PHS to include "avoid promiscuous sex" and "avoid prostitutes" as two basic messages and stresses the need for parental involvement in AIDS education. The programme should

Japan's austere 1987 budget ignores inflation

Tokyo

THE most austere budget in 32 years has been proposed for Japan in fiscal year 1987. The draft budget, approved by the cabinet, has been frozen at last year's level. Nevertheless, some large increases have been won for certain sectors of science and technology. The nuances of the financial package will be formally debated in the Diet in the next two months.

Despite a ballooning trade surplus, Japan is in debt to the tune of \$1 million million. And this year, as in the past several years, funding for the ministries has been drastically cut, except the Defense Agency which will enjoy a healthy boost in funding of more than 5 per cent.

The completion of some projects and cuts in funding for energy-related research have allowed the launching of new projects in the science-based ministries. The budget for the Science and Technology Agency, as in previous years, shows an increasing commitment to basic research as well as "big science" (such as space and nuclear power). "Frontier Research", a 10-year international basic research programme to investigate homeostasis and develop "frontier materials" (for example, biochips) gets a massive boost. The Ministry of International Trade and Industry (MITI) has also more than doubled its funding for biochip research in its programme "basic technology for future industries", and several large electronics companies are showing interest in this field of the future.

The completion of the MOS-1 marine observation satellite, due to be launched next month, has released large amounts of funds for development of the engineering test satellite ETS-VI, due for launch in 1992. The H-2 rocket also gets a huge infusion of funds, taking up nearly a third of the agency's space budget. There is also a 50 per cent increase in outlay for Japan's module for the US space station.

The large increase in ocean research funding is to cover the costs of building the new deep-sea submersible *Shinkai 6500* and its mother ship. Similarly, the large outlay for marine science in the budget for the Ministry of Education, Science and Culture is to cover building a new research vessel for the Ocean Research Institute at the University of Tokyo.

In line with the Education Reform Council's recommendation to "internationalize" the education system, the ministry has increased the number of foreign students it supports with scholarships to well over 4,000. The target for the 21st century is a foreign student population of 100,000, with 10,000 supported by scholarships.

Since the early 1980s, the ministry has been trying to increase cooperation in re-

search between the public and private sector with small success (although the funds involved are minuscule compared with the research and development outlays of industry as a whole). To encourage coopera-

Japanese science budget

Science and Technology Agency	Yen (thousand million)	Percentage increase or decrease
Total research and development budget	432.5	+ 1.1
Special promotion funds	8.5	+ 8.7
Space	94.6	+ 2.1
Nuclear energy	273.4	- 0.7
Ocean research	7.7	+ 16.2
ERATO	3.2	+ 17.5
Frontier research	1.5	+ 37.2

Ministry of Education, Science and Culture

Grants-in-aid of research	45.1	+ 3.7
Maintenance of scientific research system	8.0	+ 17.2
Research fellowships	1.1	+ 49.5
Energy	13.4	- 10.2
(Nuclear fusion)	(7.6)	(- 7.0)
(New energy sources, energy conservation)	(5.7)	(- 14.0)
Accelerator physics	13.2	-
(TRISTAN)	12.9	(- 0.9)
Space science	11.8	- 4.4
Marine science	3.0	+ 470.8
Earth science	2.1	+ 0.5
Antarctic research	2.9	- 5.5
Cancer research	1.8	+ 4.8
International academic exchange	3.6	+ 3.7
International student exchange	14.5	+ 23.9
Japanese language education	0.3	+ 8.3

Ministry of International Trade and Industry

Total technology development budget	196.4	- 0.3
Basic technology research promotion centre	25.0	+ 22.0
Basic technology for future industries	6.0	- 8.3
Large-scale industrial projects	15.1	- 0.7
Sunshine project	44.1	+ 2.6
Moonlight project	11.4	- 7.3
Fifth generation computer project	5.6	+ 1.8
Unmanned space platform	1.7	+ 850.0
Laser enrichment of uranium	4.3	-
Proving tests for technology for fast breeder reactors	4.0	-
Human frontiers science programme	0.2*	-

* Includes Y150 million from the special promotion funds of the Science and Technology Agency

tion and direct research towards the practical needs of society, "joint research centres" will be set up in some universities, and the first three will open in 1987 at Kobe, Toyama and Kumamoto universities with funding for "maintenance of the scientific research system". Funds from this category are also being used to establish an "International Center for the Study of Japanese Culture" in Kyoto, which will be a National Research Institute for joint university use.

Although there is a decrease in outlay for nuclear fusion, the ministry is still committed to moving Nagoya University's Institute of Plasma Physics to a new site in Gifu Prefecture (see *Nature* 320, 204; 1986), and the last parcel of land for this will be bought and levelled this year.

The education ministry's Institute of Space and Astronautical Sciences (ISAS) has won approval for its plan to launch the Solar-A satellite in 1991 to observe solar flare activity with two X-ray telescopes to be developed jointly with US scientists (see *Nature* 322, 762; 1986). And, according to Minoru Oda, director of ISAS, UK space scientists will also join the project.

ISAS is also involved in plans to develop an unmanned space platform in collaboration with the Science and Technology Agency and MITI; MITI's budget for the platform this year has increased tenfold to Y1,700 million. The platform, to be launched in 1992, probably by an H2 rocket, will perform engineering tests (for example solar power generation) and may carry a medium-sized infrared telescope.

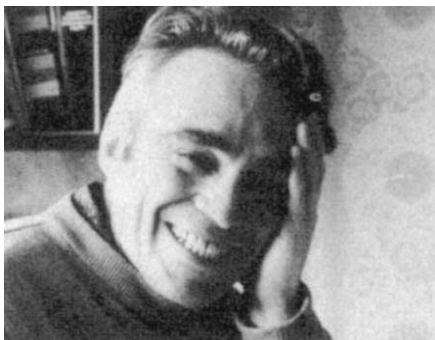
MITI has raised Y8,300 million to begin development of techniques for the laser enrichment of uranium and to carry out proving tests of components of fast breeder reactors. Japan was caught by surprise in 1985 when the United States announced its intention to adopt the laser method, but MITI and the Science and Technology Agency now have well-funded programmes for the technique.

Two hundred million yen has been set aside by MITI and the Science and Technology Agency to launch the Human Frontiers Science Program, a major international research project in basic bioscience. Scientists from Europe and the United States have been invited to Japan to discuss the programme with ministry officials and academics. MITI's plan calls for the establishment of a non-profit foundation which will accept funds from the Japanese government, industry and abroad. The foundation will then channel funds to the "Center for the Promotion of the Human Frontier Science Program", which will disburse grants to participating researchers, universities and institutes, in Japan and overseas. **David Swinbanks**

Moscow refusniks suffer over *Nature* letter to scientific union

London

A GROUP of Moscow refusniks who published a letter recently in *Nature* (324, 204; 1986) report reprisals from the KGB and Public Prosecutor's Office. The letter was addressed to Professor Jean-Marie Legay, president of the World Federation of Scientific Workers (WFSW), and two of the signatories, Dr Alexander Ioffe and Dr Boris Klotz, had tried to deliver it to Legay personally last July, during the WFSW General Assembly in Moscow. They were, however, prevented by plainclothes men from meeting Legay in his hotel, although he had agreed to a meeting. Unable to arrange another meeting, they decided to convey their message to Legay through the columns of *Nature*.



Ioffe — managing to smile through.

The letter drew attention to the position of refusnik scientists prevented from emigrating from the Soviet Union to Israel, and yet, while in the Soviet Union, deprived of any real possibility of continuing their professional activity. They noted the many efforts on their behalf by the world scientific community, and called on WFSW to join these efforts.

Following the appearance of the letter, four out of the nine signatories reported further harassment: Victor Fulmacht was told that the refusal of his emigration visa was "final" and that further applications would not be considered; Dr Mikhail Kholmyansky was summoned to the Moscow Public Prosecutor's Office and interrogated about the letter in *Nature*; and Klotz was similarly interrogated, in his case by the KGB, which wished to know who had written the text and under what circumstances. The administrations of the institutes where Ioffe and Klotz are employed inaugurated procedures which seemed aimed at their dismissal. For a time, Ioffe's salary was withheld, without legal justification.

Contacted last week, Legay said he was unaware of the appearance of the letter in *Nature*. After reading it, he said that he took exception to one expression in it: the exhortation to WFSW to "join" the efforts on behalf of the refusniks. WFSW, he

said, has for many years worked vigorously for the rights of scientists — he instanced its work for Argentinians and the victims of West German *Berufsverbot*, and reiterated his willingness to help the refusniks. But WFSW, he said, could only work within its constitution, that is, by

behind-the-scenes "quiet diplomacy".

As for the aborted meeting in Moscow, Legay explained that after the refusniks had been prevented from meeting him in his hotel, they had telephoned him to suggest another appointment elsewhere. But this was impossible, he said, because of his position as president of WFSW. Not only did he have no time during the general assembly to go off to such a meeting; it was also "undesirable" for someone in his position to do so.

Vera Rich

Pressure for SDI deployment increases in United States

Washington

THE Strategic Defense Initiative (SDI), President Reagan's plan to erect a "peace shield" against ballistic missiles over the United States, could become a reality by the middle of the 1990s, although it is still in its research phase.

Various reasons are given for this new impetus, with congressional unwillingness to spend large sums on the research programme conspicuous among them. Last year the administration saw its \$5,400 million SDI budget request cut to \$3,530 million. The administration is seeking \$5,800 million for the programme this year, but observers doubt whether much more than \$3.5 million will be forthcoming. Senator Dan Quayle (Republican, Indiana), a staunch supporter of SDI, believes Congress is losing patience with SDI research, and has urged the White House to consider some sort of phased deployment.

According to press reports, that is just what it is doing. A meeting apparently took place last month where alternatives were presented to President Reagan. But officially nothing has changed.

Different schemes have been proposed to phase in SDI. On one, developed by the George C. Marshall Institute, a decision would be made this year to permit an initial phase to be deployed by 1992. The first step, according to the Marshall Institute plan, would be a rapid deployment of an Exoatmosphere Re-entry Vehicle Interceptor Subsystem (ERIS). Together with a boost phase defence based on Space-Based Kinetic Kill Vehicles (SBKKV), and a terminal phase using the High Endoatmospheric Defense Interceptor

(HEDI), the report concludes that a three-tiered system could be in place by 1994.

To achieve 93 per cent effectiveness against a Soviet launch force of 1,200 Inter-Continental Ballistic Missiles (ICBMs), 11,200 warheads and 90,000 decoys, the report calculates that 11,000 SBKKVs, 10,000 ERIS interceptors and 3,000 HEDI interceptors would be needed. The effectiveness would increase to 99 per cent if the ERIS layer were assumed to be able to identify decoys. The authors of the report say a fully operational system would cost \$121,000 million.

But Richard Garwin of IBM's Thomas J. Watson Research Center, a critic of SDI, is unimpressed with the institute's plan. He feels the report is optimistic, both in the numbers of Soviet decoys and the effectiveness of the SDI defences.

Secretary of Defense Caspar Weinberger was repeatedly questioned last week about the administration's plans for SDI. In a speech at the National Press Club, he said the administration was exploring four or five ways of deploying SDI but was not committed to a firm timetable. Weinberger said he did not think a partial deployment of SDI would harm chances for an arms control agreement at the talks now in progress in Geneva, taking the view that progress on SDI gives the Soviet Union an incentive to agree to arms control.

The Soviet Union has so far shown no sign of changing its position on SDI. H. Allen Holmes, assistant Secretary of State for politico-military affairs, says the Soviet Union is still formally insisting that SDI should be only a laboratory project.

Joseph Palca

Boost Phase	Space-Based Kinetic Kill Vehicles (SBKKV)	Kinetic energy weapons, launched from satellites, fired at boosting missiles and buses deploying warheads in post-boost phase.
Midcourse Phase	Exoatmosphere Re-entry Vehicle Interceptor Subsystem (ERIS)	Ground-based kinetic weapons Airborne Optical System track warheads and direct weapons
Terminal Phase	High Endoatmospheric Defense Interceptor (HEDI)	Heat-seeking missiles to intercept incoming warheads at low altitudes

First human AIDS vaccine trial goes ahead without official OK

London

A CONTROVERSIAL trial of an AIDS (acquired immune deficiency syndrome) vaccine in Zaire has been given the full backing of the Zairean government in the face of mounting criticism that it is being carried out secretly and without the backing of the World Health Organisation.

The vaccine, which is the first to be tried in humans, has been developed by Dr Z. Lurhuma, director of the immunology laboratory of the University Clinic in Kinshasa, General J.-J. Salaun, head of the National Institute of Biomedical Research in Kinshasa (formerly the Pasteur Institute and now operated with French cooperative aid), and Dr Daniel Zagury of the Université Pierre et Marie Curie.

Zagury's research, much of which has been in cooperation with Dr Robert Gallo, has led him to design a vaccine aimed to trigger cells of the immune system to kill cells that are infected with the AIDS virus and carry viral envelope protein on their surface. Because the killing is mediated by cells of the immune system as well as by virus-neutralizing antibodies, the prototype vaccine has a different, but still undisclosed, basis from that of the several candidate vaccines that are based on purified envelope protein.

Animal tests followed by tests on a few uninfected human volunteers have been

used to show that the vaccine is free of toxicological effects and can stimulate antibody production. As a result, the vaccine is now being tested on a small scale in volunteers at high risk in Zaire.

Zagury and his colleagues are not prepared to comment on the results of the trial so far, preferring to await its completion and formal presentation. They are, meanwhile, delighted by the statement of support and encouragement for the project from the Executive Council of Zaire that was published in *Elima*, the main Kinshasa newspaper, on 7 January. The statement looks forward to large-scale trials.

With a high incidence of AIDS and a correspondingly high risk of infection in Zaire, a large-scale trial of the vaccine should provide a trial of efficacy in a relatively short time. The rates and risks of infection in Zaire have been extensively documented as the result of an international project backed by the US Centers for Disease Control. But it seems clear that the project fell far short of the hopes of those in Zaire and that a vaccine trial is much more to the point. And they are disinclined to involve the World Health Organisation's Control Programme on AIDS headed by Dr Jonathan Mann, who until recently was deeply involved in the Centers for Disease Control project in Zaire.

Peter Newmark

First Chinese scientist in hot water

London

THERE is consternation among theoretical astrophysicists in the West, and especially at Princeton, that Professor Fang Li Zhi should have been one of the first casualties of the response of the government of China to the student demonstrations of recent weeks. The Chinese authorities announced last week that Professor Fang, who was vice-president of the Hefei University of Science and Technology in Anhui, was said to have been expelled from the Chinese Communist Party. Other reports said that Fang had been assigned to work at the observatory of the Chinese Academy of Sciences at Beijing.

Fang has been prominent among Chinese scientists collaborating with those overseas since the opening of China to the West a decade ago. For the first half of 1986, Fang worked at the Institute of Advanced Study at Princeton with the help of a fellowship under the scheme for scientific exchange between the United States and China which is administered by the US National Academy of Sciences.

Fang's colleagues at Princeton describe him as an able and imaginative scientist. Most recently, he appears to have been

working on models of the Universe incorporating cosmic strings. There had been plans for continuing collaboration between US and Chinese theorists in the field, although it is not yet clear whether these will materialize in what may be a more chilly climate.

The University of Science and Technology is unusual among Chinese universities in being founded by the Chinese Academy in 1959. It was moved from Beijing to Hefei, in Anhui province, ten years later, when the cultural revolution was at its height. By Chinese standards, Hefei is comparatively small, with about 3,000 undergraduates, most of them on five-year courses.

The official announcement of Fang's dismissal last week said that he had been drummed out of the Communist Party for having advocated "bourgeois liberalism" and for having encouraged students to demonstrate in favour of what they called "greater democracy" during the past four turbulent weeks. The obvious fear is that Fang's departure may mark a return to the suppression of Chinese intellectuals, with scientists prominent among them, that distinguished the Cultural Revolution. □

NRC panel finds regulations on export too broad

Washington

ATTEMPTS in Congress to liberalize labyrinthine US export regulations are likely to be boosted by a controversial study made public last week by the National Research Council. The study, by a blue-ribbon panel chaired by Lew Allen Jr, former director of the National Security Agency and director of the Jet Propulsion Laboratory, supports the view common among high-technology industries that US

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Thiel photography

Lew Allen Jr — liberalizing regulations?

attempts to deny the Soviet bloc access to militarily important technology are over-zealous. It concludes that the regulations — "not generally perceived as rational, credible and predictable" — encompass too many products and technologies to be administratively feasible, and estimates that US industry would gain about \$9,000 million per year in exports and 200,000 jobs if the United States adopted the same policies as its allies in the North Atlantic Treaty Organisation (NATO).

The United States is the only Western country that imposes unilateral controls on exports that are more stringent than those agreed jointly by member nations of CoCom, the informal Coordinating Committee on Multilateral Export Controls consisting of NATO members (except Iceland) and Japan.

In particular, US attempts to require overseas recipients of US technology to certify they will not re-export sensitive items have caused resentment among allied nations. The allies claim that such "extraterritorial" provisions violate international law. The Allen panel recommends that the United States restrict its controls to CoCom-proscribed items, and to those destined for a proscribed country or for one that has not agreed to abide by CoCom rules.

Allen's critique is a slap in the face for Pentagon hardliners who have pressed for tighter export regulations. One such architect of defence policy, assistant secretary of defence Richard N. Perle, hit

back last week by accusing the study of having been conducted by "interested parties... predominantly representing the business community". But although several panel members have a business affiliation, the national security panel is also well represented. Allen was formerly chief of staff of the Air Force, and the panel included several other defence luminaries, not least Admiral Bobby Inman, another former director of the National Security Agency who was one of the first government officials to voice concern about the "haemorrhaging" of Western technology to the Eastern bloc.

The Pentagon had already showed its contempt for the study in February 1986, when it withdrew its cooperation after liaison was switched from the Pentagon's department of research and engineering to its policy-making division. It then refused to pay the second of two agreed \$100,000 contributions towards the cost of the study. A Pentagon spokesman said the decision was taken because the study was not producing new information or practical recommendations, and pointed out that no other government agency (such as the Commerce Department, criticized in the study for being ineffective) had provided more than the Department of Defense (DoD) contribution.

Perle's thesis is that tighter controls are necessary because many Soviet military advances have demonstrably led straight from technology acquired illicitly from the West. Allen's report does not dispute the premise, but argues that current regulations impose trade barriers on many items that, although militarily useful, can easily be acquired from third countries. The Pentagon weakened its case by failing to provide data in support of its estimates of the cost to the West of Soviet acquisition of Western technology.

The other side of the coin is trade: because of the complexity of the US export licensing system and the uncertainty and delay it engenders, US industry complains it is losing orders to overseas suppliers. Allen's panel, which was able to analyse the Department of Commerce's administration of export licence applications, concluded that such self-imposed trade barriers threaten US — and the West's — technology advantage over the Soviet Union by stifling innovation. Despite some attempts recently by the Commerce Department to be more responsive to industry's needs, DoD still exercises a veto which in practice causes frequent delays for companies wanting to export to certain countries. By restricting US exports, the panel found US regulations are encouraging the growth of overseas sources for so-called "dual use" items with military and civilian applications.

The solution the Allen panel proposes is to strengthen CoCom and to limit controls to truly critical items. One of the problems

of the present unilateral US control system is that there is no effective way of removing items from the "control list" once they have been put on it — and it apparently includes almost every modern industrial process. The Allen panel suggested that developing countries with rapidly growing technological capabilities be encouraged to institute "CoCom-like" procedures.

Allen's panel expresses concern about several recent moves by DoD which place under tighter control technical information arising from its research projects, even when such information is not classified. The panel is particularly concerned

about the impact of such new control schemes on professional societies' meetings; it believes the benefits of open dissemination of research data outweigh possible risks.

Some observers believe that, as a practical matter, Allen's report, already publicly dismissed by DoD, will have no effect on bringing about change in US policy. But others, including some Capitol Hill staff, are not so pessimistic. There is, they point out, likely to be much interest in US economic competitiveness in the 100th Congress, and far-reaching trade legislation is on the agenda. The research council's study may yet pay off. **Tim Beardsley**

UK long-term public spending plans turn the clock back 20 years

London

THE British government intends further to reduce the amount of public spending, in proportion to national income, over the next three years and to demand greater value for money from its investments, including scientific research. Researchers on government projects will receive a modest increase in funds in the next year — in the run-up to a general election — but the rate of growth will be quickly curtailed in the following year. Public spending in 1987–88 and 1988–89 will be £148.6 and £154.2 thousand million, respectively, with a new spending limit of £161.5 thousand million set for the following year. By the end of the decade, if the plan is not altered, Britain will have turned the clock back nearly twenty years on public spending.

Specifically, what this means is that the budgets of most spending agencies, measured in strictly cash terms, are likely to increase more quickly this year than next, at least on present plans. Thus the total budget of the research councils, dependencies of the Department of Education and Science, are to increase by about 6 per cent between this year and next (beginning 1 April), but then by only two per cent in the succeeding year. Much the same applies to the budget of the University Grants Committee, although the planned reduction between 1987 and 1988 will not be so sharp.

The government has made little secret in recent years of its discomfort about the level of public expenditure. Its investment in scientific research, about £4.3 thousand million a year, is under threat as government pushes for industrial investment in research and attempts to transfer the results of research, particularly in defence, to the commercial sector. The Ministry of Defence consumes about half of the publicly funded research cake. The mood at the Natural Environment Research Council, which published its annual report

almost simultaneously with the government's expenditure plans last week, reflects that pressure. The council, which has agents around the world and conducts much work for industrial/commercial clients, intends to create its own commercial subsidiary to sell and exploit the products of its research.

Previous attempts to exploit British research commercially have depended on the creation of new bodies to help steer the research towards the commercial sector. The National Enterprise Board (NEB) and the National Research Development Corporation (NRDC) were two such bodies set up by previous administrations, the former taking substantial equity stakes in manufacturing and high technology industries.

The present government had a different approach and demanded more industrial participation and a reduction in government expenditure. It inherited NEB and NRDC, and merged them into the British Technology Group, ensuring that major government shareholdings were sold. Although the logic behind the new expenditure figures is consistent with previous government thinking, no attempt has been made to address the issues outlined in two major science plans now before ministers. The first, presented at the end of last year by a committee headed by retired Glaxo chairman Sir Austin Bide, called for about £500 million of public money to be invested in industry and scientific research, which in turn would attract the same from industry.

The second is the British 15-year space plan, presented to government last summer in anticipation of a positive response in November. The plan calls for an additional £200 million a year for space research and development — twice the current figure.

Neither plan has received a response and both were ignored in last week's budget disclosures. **Bill Johnstone**

Academies slam NRC on nuclear safety research programme

Washington

THE US nuclear safety research programme is in trouble and in dire need of reform. This is the uncharacteristically scathing criticism levelled two weeks ago in a report* by the National Research Council, the research arm of the academies of science and engineering, at the safety research programme of the Nuclear Regulatory Commission (NRC).

The authors of the report say they were shocked to learn that the director of the NRC Office of Nuclear Regulatory Research at one point had not met the commission as such for nearly two years, even though the research office controls a quarter of NRC's \$410 million annual budget.

The committee also says that coordination between the research office and the Office of Nuclear Reactor Regulation is inadequate. The committee deplores the jurisdiction disputes that seem repeatedly to erupt between the two offices and the lack of a research philosophy to guide the agency's work.

Part of the trouble seems to have stemmed from budget cuts over the past five years. NRC's statutory mandate is for the safety and licensing of nuclear reactors, which means that research has always been an attractive target for budget cutters. The committee notes that the debates over the relative contributions that should be made by government and industry to nuclear safety research have not helped, but it takes no position on the proper size of NRC's safety budget, saying merely that what money there is should be spent wisely. The committee also asks for industry participation at all stages of the safety programme, asking that NRC should find the best people to carry out its research, whether they are in industry, universities or the national laboratories.

More constructively, the report from the National Research Council makes specific suggestions to make about areas of safety research that deserve attention. It says materials research will be relevant to most areas of reactor safety, and that the need for a better understanding of human factors has been underlined by the accidents at Three Mile Island and Chernobyl.

On the development of new and advanced reactor designs, the report asks that NRC should pay attention to the sometimes novel safety considerations that arise, saying that it is "poor public policy" to let advanced reactor development to proceed without regulatory guidance.

Ordinarily, as with other NRC studies, the report would have been more con-

cerned with suggestions for research. But John Ahearne, a member of the committee responsible, says that the management of research at NRC must be sorted out before a research strategy is possible.

NRC is still studying the report, but there are signs that changes are afoot. The budget request on behalf of NRC sent to Congress earlier this month asks for an extra \$8 million for research, which would take the total for next year to \$119.7 million. Management changes have been

under way since last November, when NRC announced that its research branch would be given full responsibility for generic safety issues and for probabilistic risk assessment. The research office will also concentrate on making its research relevant to regulatory needs.

It will require strong leadership from NRC if safety issues are to receive the attention they deserve, according to the committee, whose report says that strong leadership has been lacking in the past. It says, nevertheless, that "nuclear safety research is too important to be continually whipsawed and debilitated by bad management and the vicissitudes of the political process".

Joseph Palca

Threat of imminent disruption in British university faculties

London

AN unprecedented level of university disruptions with no students being awarded a degree this summer is the threat posed by UK academic staff, dissatisfied with government's intransigence and its lack of urgency in responding to a 6 per cent wage claim, now ten months old.

The action, to be led by the Association of University Teachers (AUT), which represents nearly 50,000 staff affected by the

teachers nor the students have officially expressed their views. She says: "If Mr Baker does not immediately agree to settle the salary claim for university staff he will precipitate an unprecedented level of disruption in the universities, with no student being awarded a degree this summer. Our members are always reluctant to jump to action, but now our patience has snapped. We deeply regret the possible repercussions on individual students. The National Union of Students is sympathetic to our action, and we're seeking their co-operation in breaking through this 10 month stalemate." AUT claims its members have been pushed into this action and "must now act to focus attention on the worsening plight of universities. We call on the vice-chancellors and the government to respond now."

The university staff pursued a parity claim last year, maintaining that their salaries had fallen 44 per cent behind their industrial equivalents (at the age of 32 years) and nearly 20 per cent more behind the 'high fliers'. The claim was for 6 per cent from April last year and another 18 per cent from April this year.

But the University Authorities, AUT says, refused to pay the 6 per cent and the education secretary "has reneged on his undertaking to indicate his response to the erosion and the restructuring claim".

There are no indicators that can accurately predict the support which the teachers and the students will command in any industrial confrontation. But the education secretary is not easily intimidated. He has already introduced legislation to abolish the body, the Burnham Committee, established under a previous administration to settle teachers' pay. After about two years of disruptive action in Britain's schools over pay and working conditions, the education secretary introduced legislation to impose a settlement on the disgruntled school teachers. **Bill Johnstone**

IMAGE
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Diana Warwick claims student support.

claim, will ban examination marking, prevent the award of degrees and "totally disrupt the graduate recruitment programme by industry and commerce and throw university administration into chaos".

AUT accuses the Secretary of State for Education and Science, Mr Kenneth Baker, of "cynical tardiness" in his reluctance to disclose what money has been allocated to university salaries.

The association is to conduct two national ballots, the first on a one-day strike on 5 March and the second to endorse or reject the non-marking strategy.

Diana Warwick, general secretary of AUT, claims also to have the support of the student body, although neither the

* *Revitalizing nuclear safety research*, National Academy Press, Washington DC, 1986.

Survey highlights racialism at top US technology institute

Boston

MINORITY students cannot turn to their professors for help because many faculty members expect such students to fail and have little confidence in their abilities. Racial prejudice among the student body adds further to the minorities' difficulties. These startling results emerge from a study conducted by the Massachusetts Institute of Technology (MIT) on its own students and published by the institute.

The study has disturbed the authorities at MIT; it shows that they have failed to create a racially open environment and that the incidence of bias is unacceptably

impossible to create a structure formally to eradicate prejudice.

Because 40 per cent of good interactions between faculty and black students was reported to have occurred with the 1–2 per cent of the faculty members who are black, an increase in black faculty could be beneficial. But, says Gray, the number of blacks in science and engineering graduate school is down; the pool of blacks at the doctoral level in relevant disciplines has not increased in 20 years.

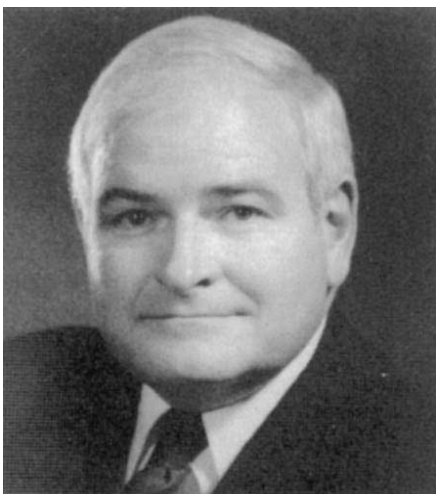
The report says that MIT's goal is an interaction between races defined as "pluralism": "Pluralism is different from . . . diversity, in which individuals from various groups are merely present, just as it differs from the idea of integration, in which minority individuals are asked, ex-

PLICITLY and implicitly, to abandon their cultural identity in order to merge into the majority community."

How to foster pluralism rather than pressure to segregate or integrate is an open question among MIT students. On an opinion sheet put up for student response, many white students objected to separate living patterns of blacks, while blacks pointed to a need to feel at home somewhere.

An increased applicant pool will help the institute reach a critical mass of minority students, says Gray, but the seesaw in numbers will create an additional problem. Standardized tests, he says, are poor indicators of individual performance but have "substantial meaning" as a group predictor. For a number of reasons, blacks and hispanics score below whites, as Asians score above. Schools "with the expectations of MIT will be in a pickle unless these students come better prepared".

Elizabeth Collins



Dr Paul Gray — willing to tackle discrimination.

high. By publishing, says President Paul Gray, the institute shows its willingness to tackle discrimination against minorities and draws attention to the problems facing predominantly white campuses.

The most disturbing behaviour, according to Gray, is the supposed reaction of faculty members to minority students. Over half the respondents to a telephone survey of black MIT graduates reported negative reactions from their faculty members. One third claimed that the staff expected failure or poor ability from them.

Many students quoted in the report echoed the same sentiment. "I went to talk to [a professor] about a grade, and he said that 'maybe you people should go somewhere and do things you people can do'". Minority students, concludes the report, soon learned not to turn to white faculty members for help. The prejudicial atmosphere is made worse by a much expressed view that high admission standards have been waived for ethnic minorities who otherwise could not have qualified for the institute.

MIT is rigorous for any student, says Gray, and although discrimination compounds the problems for minorities, it is

Hungarian investment needed in research and development

London

HUNGARY needs increased investment in research and development, according to the Central Committee of the Hungarian Socialist Workers' Party. A position paper prepared by Dr Lenart Pal, formerly chief secretary of the Hungarian Academy of Sciences and now a secretary of the Central Committee, was "discussed and approved" by the Central Committee on 28 December; it notes that in recent years, party and government decisions urging technical development have not been sufficiently implemented because of a lack of resources and the failure of economic management to make efficient use of such resources as are available. Research and development expenditure should increase, it was urged, more rapidly than national income.

Research and development financing in Hungary involves a range of economic strategies from research contracts between production enterprises and academic research institutes on a strict commercial basis to government support administered through the Central Fund for Technical Development. A feature of Hungarian research and development unique in the socialist bloc is the existence of profit-orientated research companies that can apply for government support.

On the basis of the position paper, the Central Committee considered that the profit motive should be further stimulated, with research encouraged by the "differentiated use of finances", an improved scheme of tenders and commission and the creation of competition. Producing and trading companies should "play a

determining role in setting the targets of applied research, and the evaluation, application and financing of achievements". At the same time, the scheme by which manufacturing companies now have to contribute 15 per cent of their profits to a centralized technical development fund should be abolished, and each company should be left free to decide how much of its profits to plough back into research and development.

The Central Committee also recommended the "lasting division of labour and cooperation" between the research sector (including the universities) and the production sector, to promote the rapid implementation of scientific achievements in practice. In spite of the cutbacks in the university and academy budgets over the past few years, in at least one field officially considered of paramount economic importance — biotechnology — the trend has gone the other way, and there has been a separation of the academic and applied sectors. Thus, the Szeged Biological Research Centre of the Hungarian Academy of Sciences has cloned off a new commercial biotechnological research centre now under construction on an adjacent site, while government plans announced in 1984 to develop the Agricultural University of Godollo as a major centre for agriculture-related biotechnology were found to be administratively impractical, and materialized instead into a research centre (also now under construction) close to but independent from the university, and the introduction of new, biotechnology-related undergraduate courses in the university itself. Vera Rich

Spanish astronomy defended

SIR—As a British astrophysicist who has been working in Spain during the past two years, I read with surprise and concern a statement in your "Science in Iberia" issue (*Nature* 324, 318; 1986) that "Astronomy appears to be a major disappointment. Despite access to the superb international facilities in the Canary Islands, very few Spanish papers appear in top journals."

This statement, insofar as it bears any relation to the real world, must be based on an outdated and therefore highly misleading set of facts. The facilities referred to were inaugurated formally in 1985, have been in partial operation since 1984 and will not come into full operation until the late 1980s. Anyone with even a limited understanding of research knows that publications do not appear instantly when experiments or observations are performed. *A fortiori* this cannot be expected to occur if there is a need to train a first generation of scientists almost from scratch before they can participate in the activities of acquisition and reduction of data, and subsequent physical interpretation. This has been the situation with Spanish astrophysicists in general, and with the Instituto de Astrofísica de Canarias (IAC, which has administrative charge of the Canary Observatories) in particular.

One would expect the publication rate to rise significantly once the first 'crop' of PhDs from research students trained in both non-Spanish and in Spanish institutes begins to turn out independent work, probably next year. It is therefore to the credit of the young Spanish astronomical community (and to the foresight of those few who planned ahead) that it has already begun to make an impact. A clear indication of this was the initiative of the editorial board of *Astronomy and Astrophysics* to invite Spain to become a member of this leading European journal, given the recent sharp rise in the number of Spanish publications. In 1986, for example, 15 papers from this institute alone were published in *Astronomy and Astrophysics*, which may be compared with the 13 from the Max Planck Institut für Astronomie at Heidelberg and 31 from the Institut d'Astrophysique in Paris, an institute with more than twice the staff of IAC. Further, it is acknowledged within the UK Science and Engineering Research Council that the quality of Spanish applications for time on the Canary Island telescopes is as high and the oversubscription rate as great as those of applications from other members of the observatories' international partners, which include the United Kingdom. I would personally wager that if present funding levels for astronomy here are not reduced, Spain will become one of the four or five leading

nations in Europe within 20 years — a short time to build a tradition.

It is possible that the author of the remark quoted above was misled by a recent study in *Mundo Científico* (62, 1002; 1986), the Spanish edition of *La Recherche*, giving figures about astrophysical publications from Spanish institutes. This study was based on data collected before the Canary Observatories were in operation, and has in any case been severely criticized for omitting many publications from Spanish astrophysicists in international laboratories.

JOHN E. BECKMAN

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SIR—In *Nature's* special issue about "Science in Iberia" the following statement appears (324, 318; 1986): "Astronomy appears to be a major disappointment. Despite access to the superb international facilities in the Canary Islands, very few Spanish papers appear in top journals."

This declaration is as serious as it is frivolous and it would be very interesting to know the source of such a negative idea.

The "superb international facilities in the Canary Islands" were inaugurated just last year by the King and Queen of Spain. While some of the telescopes commenced part-time operation in 1984, others will not even see first light until 1988 so it would surely be premature to expect abundant "Spanish papers in top journals".

The real state of affairs is that Spanish astrophysics, non-existent only 20 years ago, is beginning to enjoy a promising prestige among the international scientific community. Only 15 years ago, there was not even one chair of astrophysics at any Spanish university; now there are more than 25 professors of astrophysics. The number of PhDs in this field is over 50 and the universities produce more than 40 MAs in astrophysics each year. Whereas there were no institutes of astrophysics, there are now two, and nearly one hundred papers are published in international journals every year. What is more, we are expanding rapidly and production is increasing daily. But the most promising feature is that the brightest youngsters are becoming increasingly interested in astronomy.

This great leap forward has been achieved by the enthusiastic work of a handful of Spaniards backed by our European colleagues and greatly helped by international investments. Focusing, as an example, on the Canary Islands' observatories, thanks to the 20 per cent observing time plus the 5 per cent for projects in collaboration that we receive free of

charge, young Spanish research groups are managing to observe with the finest modern instruments while mixing with the world's top astrophysicists. The final launching of modern astronomy in Spain is simply a matter of time. The 'Solera' (the distinctive flavour of a mature sherry) that we have lacked in astrophysics is being gained very rapidly.

Even the Minister of Education and Science and the Secretary of State for Universities and Research are well aware of the privileged situation of astrophysics in our country and their intention to pay it special attention is well known.

FRANCISCO SANCHEZ

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Civil servants

SIR—In clarification of your story about the Plant Gene Expression Center (*Nature* 324, 103; 1986), Peter Quail will be an employee of the University of California in Berkeley, but our other six newly named staff members will be federal civil servants. Because the centre is a cooperative venture with the University of California system, these federal employees will probably also have faculty appointments at the Berkeley campus. Other scientists within the Agricultural Research Service have adjunct appointments with local universities.

GERALD G. STILL

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Agricultural Research Service,
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Gene Expression Center,
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Too old at 30

SIR—The rendering by Bill Johnstone (324, 8; 1986) of the prejudice of some faculty members (and search committees) "in favour of [the superior creativity of] people younger than 33" is not unfamiliar to me. As a postdoc over the age of 33, however, permit me to gather together the tatters of my creative insight and delicately point out that these gentle academicians could best perceive the strength of their peculiar logic if they applied it to themselves.

May one then expect to behold the amazing sight of a resignation *en masse* of the 'over-age' among these who, with self-consistent altruism, will no doubt equally put the good of scientific progress over the feeble expression of their own fast-fading creative powers?

PATRICK FRANK

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Satellite weather around the corner?

A cool appraisal of the contribution of Earth satellites to weather forecasting reveals basic and practical problems to be solved. Measuring water vapour may be the next objective.

METEOROLOGISTS, or at least those who work as weather forecasters, have something of the social status of economists. People look to them for good news and blame them when it is otherwise. So much has been clear, in the past few weeks, on both sides of the North Atlantic. On the eastern seaboard of the United States, the television weathermen forecast the Great New Year storm with all the zeal of physicians bent on avoiding a malpractice suit, and then complained when the snowfall was merely normal for the time of year. In Britain, where belief in the effectiveness of snowploughs has yet to take hold, meteorologists have been given no thanks for having forecast a spell of cold weather that should be considered normal so near to the Arctic Circle.

Will not all this change when sufficiently elaborate atmospheric circulation models have been bedded down in suitably large computers, and when the data that they use are collected by Earth satellites capable of measuring to whatever resolution may be required? Not so, it seems, to judge from a thoughtful review of the usefulness of satellite data in numerical weather prediction by R.D. Isaacs, R.N. Hoffman and L.D. Kaplan of Atmospheric and Environmental Research Inc. (*Rev. Geophys.* 24, 701–43; 1986).

Numerical weather forecasting has improved immensely in the quarter of a century since the US satellite TIROS I was launched, but the contribution of satellites has been less important than the improvement of forecasting technique. The good news is that satellites have yet to yield much of what they have always promised.

On one thing, however, there is general agreement: before TIROS I, when the data for the Southern Hemisphere were derived largely from the adventitious routes taken by ocean-going ships, prediction south of the Equator was much more hazardous than now. Imperfect though satellite data may still be, they have filled gaps that would never otherwise have been bridged.

Elsewhere, the forecasters' skill has improved dramatically if selectively. Isaacs and his colleagues demonstrate a dramatic improvement since the 1950s of the accuracy with which the National Meteorological Center (Washington, DC) can forecast the height of the 500-m bar level above North America a day and a half in advance; the success rate has gone from a third to three-quarters in 30 years as the

circulation models have improved. But the numerical weather forecasters are hardly any better now than then at forecasting precipitation a day ahead.

There are several difficulties, all of them persuasive that the promise of satellites is still as bright as in the 1950s. The most obvious is the problem that runs through the whole of geophysics — that of inferring from a set of measurements of a few quantities at a fixed point the distribution of some other quantity with yet another. Exploration geologists are forever trying to tell the structure of a sedimentary basin (and geophysicists the structure of the Earth) from measurements of the seismic signals generated by one event at a few places where there happen to be instruments.

The same inversion problem confronts those who would use measurements from a satellite of the radiation from the Earth's atmosphere as a function (but crudely) of the frequency to infer the distribution of atmospheric temperature as a function of altitude: one needs not merely measurements accurate enough to survive the computational barbarism of the computers, but also a theory of how one set of variables is related to the other. Often, of course, the measurements are made only because there is no theory in sight. Isaacs and his colleagues make the sensible point that, meanwhile, it would make sense to choose ranges for satellite measurements calculated to bear most sensitively, within the framework of what is known about the behaviour of the atmosphere, on the parameters likely to be most useful.

For the time being, the most useful measurements of the atmosphere appear to be temperature measurements. Thanks to infrared instruments based on interferometric principles (developed at the University of Chicago by W.L. Smith and his colleagues and installed on satellites such as Nimbus N), the temperature profile beneath the line of sight of a satellite can be determined with a vertical resolution of 5 or 6 km even in the troposphere. Isaacs and his colleagues make the disturbing observation that measurements of temperature in the troposphere from satellites and by radiosondes nevertheless appear to differ from each other by a few degrees. By contrast, the horizontal resolution provided from satellites is better than anything the meteorologists could imagine when all data came from radiosondes launched from fixed points on the Earth's

surface, usually twice a day.

The real trouble with satellites is that, apart from temperature, they have little to say that bears on the meteorology of weather forecasting. Vertical profiles of water vapour concentration are obviously important, if only because they are at once a driving force of the thermodynamics of the atmosphere and a symptom of its condition. The snag is that there is as yet no proven system for telling what the atmospheric profile of water vapour concentration may be. That stands out as the next important problem for the weather satellite designers.

Meanwhile, Isaacs and his colleagues have an interesting problem to tackle, whose method may be instructive in other fields. Relying on the availability of substantial computer power to replicate the results of atmospheric modelling using different data sets as starting material, they have set out to compare the outcome of numerical weather forecasts with and without the data provided by weather satellites. Vividly, they use the term "impact test" to describe what they have done. Possibly, one of these days, economists will find themselves compelled to run their economic models with and without the benefit of the wisdom they presume themselves to have added. What the meteorologists conclude is that data on the wind speed in the atmosphere, sometimes available from (ground-based) lidar measurements based on the reflection of laser pulses from airborne dust, appear to be powerful determinants of the numerical models. There is no obvious means by which data of that kind might be collected from observations from a satellite in orbit around the Earth. Maybe, some will say, there is a quite different parameter that will serve this purpose, which is another way of saying there is more physics to be done.

None of this implies that weather forecasting by numbers will soon be as precise as the calculation of the calendar, or that satellite observations have nothing to contribute to such a process. What is needed, rather, are some new ideas for the measurement of water vapour as a function of height, which will almost certainly require a new generation of experimental meteorological satellites even more massive than those now in service. If that would excuse the weathermen from their habitual excesses, the cost might be justified by the benefit. **John Maddox**

G proteins

'Wrong' subunit regulates cardiac potassium channels

Henry R. Bourne

THE report by Logothetis *et al.* on page 321 of this issue¹ contains a welcome surprise: a direct contradiction of the prevailing doctrine — rashly proclaimed in my recent News and Views article² — that the GTP-binding α chains of G proteins invariably regulate the effector enzymes or ion channels that serve as targets of transmembrane signalling by G proteins. The β and γ chains of these proteins can no longer be relegated to the relatively menial task of attaching G proteins to the plasma membrane, now that Logothetis *et al.* show that a complex of the β and γ chains, but not the associated α chain, reproduces G-protein-mediated opening of K^+ channels in patches of plasma membrane plucked from heart cells.

In the intact organism, acetylcholine acts on cardiac muscarinic receptors to open K^+ channels, thereby slowing the heart rate. Pertussis toxin treatment of heart cells prevents this physiological response^{3,4}, presumably by uncoupling the relevant G protein from stimulation by the muscarinic receptor. The uncoupling effect results from toxin-catalysed ADP-ribosylation of G protein α chains. Cardiac membranes contain at least two pertussis toxin substrates, G_i and G_o , which contain α -chains of relative molecular mass 41,000 (41K) and 39K, respectively⁵, called α_{41} and α_{39} .

Using the elegant inside-out membrane patch technique, Logothetis *et al.* first showed that acetylcholine added on the extracellular side of the patch opens K^+ channels only if GTP is present on the other (cytoplasmic) side. Then, in the absence of acetylcholine and GTP, they added pure α_{41} , α_{39} or $\beta\gamma$ complexes, all prepared from bovine brain, to the cytoplasmic side of the membrane. The $\beta\gamma$ complexes open K^+ channels with conductance and gating properties identical to those of the K^+ channels regulated by muscarinic acetylcholine receptors. In contrast, the K^+ channels are unaffected by similar concentrations of α chains, whether bound to GDP (inactive) or to hydrolysis-resistant GTP analogues (presumably 'active', by analogy to other G-protein α chains). Indeed, at concentrations consistent with their relative affinities for binding $\beta\gamma$, the GDP-bound forms of either α_{41} or α_{39} could prevent $\beta\gamma$ complexes from opening the K^+ channels.

As is often the case, surprise soon gives way to the realization that the earlier view was based on weak evidence. The α

chains are established as exclusive regulators of effector enzymes in only two cases (summarized in ref. 6): hormonal stimulation of adenylyl cyclase by G_s ; and photoactivation of retinal cyclic GMP phosphodiesterase by transducin. In the case of hormonal inhibition of cyclic AMP synthesis mediated by G_i , evidence points to direct inhibition of adenylyl cyclase by both α_i and $\beta\gamma$, as well as indirect inhibition by $\beta\gamma$ complexes that bind and inactivate α_i (refs 7,8). Now we must recognize the possibility that other pertussis toxin-sensitive hormonal responses (also summarized in ref. 6) — including regulation of neural Ca^{2+} channels and stimulation of phospholipases C and A_2 — may be mediated by $\beta\gamma$ complexes, α chains, or both.

In striking down a tenet of prevailing doctrine, the new result also raises a key question: how does $\beta\gamma$ transmit specific information? One answer invokes structural specificity of the $\beta\gamma$ complexes that communicate with K^+ channels. The available evidence (see refs 2, 6 for summary) argues against this notion, although weakly. Structural diversity of either β or γ (two known representatives of each) is much less obvious than that of the α chains (eight distinct chains, and still counting). Gel electrophoresis of G proteins obtained from some tissues does show two immunologically distinct β polypeptides, of relative molecular mass 36K and 35K, although their functional significance is unknown. The $\beta\gamma$ complex of retinal transducin contains only the 36K band and a γ chain that is quite distinct from that of the other G proteins. Transducin-derived $\beta\gamma$ complexes, however, are functionally interchangeable with G_i -derived $\beta\gamma$ in at least one case, when they promote the interaction of transducin α chains with photorhodopsin⁹. It will be interesting to see whether the transducin $\beta\gamma$ complex can also regulate cardiac K^+ channels. As a second test of the structural hypothesis, one might ask whether pure α_i , like α_{41} and α_{39} , can block $\beta\gamma$ -induced opening of K^+ channels. Blockade by α_i would be the expected result, because α_i can associate with $\beta\gamma$ complexes derived from G_i (ref. 8). The opposite result, however, would suggest that K^+ channels are regulated by a specific subset of $\beta\gamma$ complexes that cannot interact with α_i .

The need for conferring specificity on $\beta\gamma$ interactions with K^+ channels is specially urgent in the heart, where noradre-

naline acts by binding to β -adrenoceptors to induce G_s -mediated stimulation of adenylyl cyclase; the resulting rise in cellular cyclic AMP accelerates heart rate by an effect on membrane Ca^{2+} channels. According to a second doctrinal tenet^{2,6,8}, activation of all G proteins by GTP and the appropriate receptor results in dissociation of $\beta\gamma$ from α chains. If so, what prevents noradrenaline from slowing, rather than increasing, heart rate through K^+ channels opened by $\beta\gamma$ complexes derived from G_s ?

It may turn out that the $\beta\gamma$ complexes linked to K^+ channels and those of G_i are identical. If so, the most obvious way to prevent mixing of signals triggered by noradrenaline and acetylcholine would involve physical separation of G proteins and other signalling components in the plane of the membrane. Such separation might be achieved by organizing each signalling system into a physically distinct macromolecular array; arrays might be kept separate by the tethering of a key transducer component to the cytoskeleton. Evidence consistent with the existence of such macromolecular arrays comes from studies of the adenylyl cyclase system, including kinetic modelling of hormonal activation¹⁰, radiation inactivation¹¹ and co-purification of α_i in detergent solution with effector¹² or receptor¹³ components. Evidence for cytoskeletal attachments of these transduction systems is suggestive, but also not compelling: differential detergent extractions show that α_i in erythrocytes¹⁴ and $\beta\gamma$ in S49 cells¹⁵ fractionate preferentially with filamentous actin, a major component of the cortical cytoskeleton.

Physical separation of signalling systems can prevent mixing of signals without invoking structural differences among $\beta\gamma$ components, but only if a macromolecular complex allows the correct receptor to talk to the correct effector. The highly diverse α chains could provide the specific structural information that allows initial aggregation of the relevant G protein with a limited subset of effector and receptor molecules. Thus, α and $\beta\gamma$ subunits would become permanently attached to each other and to the effector. Here new difficulties arise: first, it is difficult to understand how exogenous $\beta\gamma$ opens K^+ channels in the membrane patches studied by Logothetis *et al.* Why do the K^+ channels, apparently dormant in the arms of one partner, suddenly begin dancing when new partners cut in? Second, aggregation of α and $\beta\gamma$ with a single effector seems to disallow a major potential advantage of using $\beta\gamma$ as a regulator of an effector molecule — the ability of a single G-protein molecule to coordinate regulation of two different targets. In addition to opening K^+ channels, for example, acetylcholine inhibits cardiac adenylyl cyclase; both effects are blocked by pertussis

toxin¹⁰. It is attractive to imagine that these complementary effects of acetylcholine result from interaction of ligand-muscarinic acetylcholine receptor with a single G protein; the $\beta\gamma$ complex of G_i could mediate the first effect, whereas GTP-bound α_i chain could mediate the second.

In summary, the experiment of Logothetis *et al.*, like all good experiments, raises a host of new questions. The rules that govern macromolecular assembly and cytoskeletal restraints on movement remain mysterious even with respect to membrane proteins much more abundant than components of G-protein signalling systems, and are objects of intense investigation in many laboratories. It seems reasonable to hope that investigators will dispel some of the mystery by exploiting the strikingly interdependent and easily measurable biochemical activities of G proteins, their receptors and their effectors. □

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Antarctic ozone

Causes and effects of a hole

J.J. Margitan

RECENT measurements in Antarctica have added considerably to our knowledge of the Antarctic ozone hole first identified by the British Antarctic Survey¹. The cause and significance of the hole remain a mystery, however, and although it is a real phenomenon, the implications for global ozone will not be clear until the mechanism by which it is produced is conclusively identified. So far, none of the proposed explanations, ranging from the effects of chlorofluorocarbons (CFCs) to a natural dynamical process, can be excluded.

Preliminary results from the US National Ozone Expedition (NOZE) to McMurdo Station, Antarctica, were announced via telephone link from Antarctica in October 1986. Observations between August and October had sought to differentiate between the three major theories that explain the formation of the hole: (1) a purely dynamical mechanism² in which rising air motions within the polar vortex lead to reduced column densities of ozone; (2) a solar activity theory³ that odd-nitrogen produced at high altitudes is transported downwards, leading to enhanced odd-nitrogen catalytic cycles that destroy ozone; and (3) several related theories in which ozone loss is caused by enhanced concentrations of odd-chlorine resulting from unusual heterogeneous chemistry associated with polar stratospheric clouds. The ultimate source of the chlorine in these theories is manmade CFCs^{4,5}.

The NOZE team operated four experiments: balloon ozonesonde and aerosol

counters (D. Hofmann, University of Wyoming); microwave radiometer (R. deZafra and P. Solomon, State University of New York; A. Parrish, Millitech); ultraviolet spectrometer (S. Solomon and G. Mount, National Oceanic and Atmospheric Administration Aeronomy Laboratory, NOAA); and infrared solar absorption spectrometer (G. Toon and C.B. Farmer, Jet Propulsion Laboratory). The McMurdo site offered the opportunity to make observations both in and out of the hole as the polar vortex shifted around the pole. Satellite data from the Nimbus 7

Total Ozone Mapping Spectrometer confirmed the appearance of the hole in early September, with a maximum ozone depletion similar to but slightly less severe than that in 1985. The NOZE ozonesonde measurements showed significant vertical structure to the hole, with 80 per cent depletion in some of the 1-km layers but only 20 per cent in adjacent layers. The depletion was confined to the 12–20-km region, beginning first at the higher altitude and progressing downwards.

The team believes that this layering is caused by evaporation of polar stratospheric clouds. The layering, and the altitude region of the depletion, are taken as strong evidence against the solar activity theory, which would have predicted depletion at high altitudes (greater than 30 km) and moving down. Only very preliminary information on other chemical species is available, however, and more detailed analyses of data from the other instruments are needed. The infrared instrument obtained high-resolution data for more than 20 days that will require computer analysis. These data are expected to provide information on the stratospheric concentrations of ozone, nitrous oxide, nitric oxide, nitrogen dioxide, nitric acid, hydrochloric acid, chlorine nitrate, methane and CFC-11 and 12, both within and outside the polar vortex.

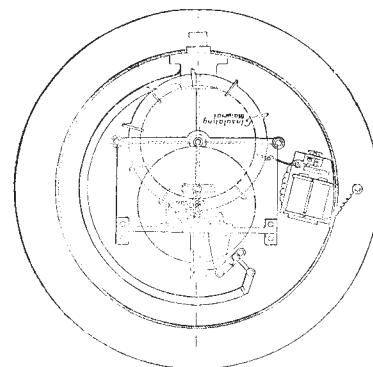
The microwave instrument produced data on hydrogen cyanide, ClO and nitrous oxide, but the high pressures in the crucial altitude region (below 25 km) greatly reduced sensitivity and so further analysis will be necessary. Preliminary results indicate, however, that the concentration of ClO is no greater than 1 part in 10⁹, not inconsistent with the halogen mechanisms but excluding the upper end of their calculated ClO concentration ranges (0.5–2 parts in 10⁹). The nitrous oxide data show unusually low concentrations within



100 years ago

A most interesting experiment is about to be tried in Birmingham. A Company has obtained Parliamentary powers to supply power from a central station by compressed air through pipes laid in the streets. No fairer field for such an experiment could be found than in Birmingham, which is marked out from all other towns by the enormous number of its small workshops requiring minute amounts of driving-power, and the total turn-over of each of which is too small to enable the owner to afford skilled tendance to his boiler and engine. In these small shops the power is required only intermittently throughout the day. The registrations of all the meters in the whole district are telegraphed to the central station and added up on

one large central counter, so that the engineers in charge may have means of continually comparing the actual consumption with the duty of the engines, known from ordinary engine continuous counters, and of detecting any serious leakage that might occur in consequence of breakage of the main or branch pipe. The telegraphing apparatus is shown.



From *Nature* **35**, 278; 20 January 1887.

the vortex, which was taken as evidence against the dynamical theory of upward air motions. Measurements of aerosol particle densities also show no upward transport. Caution is necessary in interpreting these results, however, because observations at only one location are generally inadequate for ascertaining air motions. This is especially true for McMurdo, which is near the edge of the vortex.

The NOAA ultraviolet instrument obtained data on ozone, nitrogen dioxide, nitrogen trioxide and chlorine dioxide. The ozone data are consistent with the ozonesondes and satellite data, and the NO₂ data show, within the vortex, the lowest concentrations of NO₂ observed by this instrument anywhere. These low concentrations contradict the solar activity theory, are consistent with the halogen theories and seem to be confirmed by preliminary analysis of Stratospheric Aerosol and Gas Experiment II data from the Earth Radiation Budget satellite (M.P. McCormick, personal communication). They do not, however, represent proof of the halogen theories.

The tentative conclusion of the NOZE team is that there is strong evidence against the solar activity theory and some evidence against the dynamical theory; it therefore favours a chemical mechanism as the most likely cause of ozone depletion in the hole. But data from several of the instruments have not yet been completely

analysed, so a final judgement cannot yet be made.

Indeed, results published since the NOZE announcement (but not including any of the NOZE data) in a special issue of *Geophysical Research Letters* (13, 1191–1326; 1986) led the editors of the issue to conclude that a dynamical mechanism is more likely. The issue includes more than 40 papers on Antarctic ozone, and its editors, M.R. Schoeberl and A.J. Krueger, attach particular importance to observations that significant changes have occurred in the temperature structure of the atmosphere in the region, which mirror the changes in ozone. During the August–November period, when the hole forms within the polar vortex and then disappears with its break-up, the total amount of ozone southwards of 44°S is conserved: the ozone ‘high’ increases by an amount commensurate with the decrease in the vortex. Clearly, the last word has not yet been said. □

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Evolution

An asymmetrical view of fitness

J.S. Jones

EVOLUTION is often described as the movement of populations through an adaptive landscape¹: the mathematical nature of much of population genetics theory means that this is only too often a landscape with figures. The allegorical terrain is usually hilly, with most evolving populations at or near a higher or lower summit of adaptation and able to reach a higher peak only by passing through a valley of relative unfitness. Some of these are deep and almost impassable, separating differently adapted species. On each of the major eminences are smaller summits, and as evolution is by its nature opportunistic and responsive only to an immediate challenge, populations may become marooned on a low hillock when higher peaks are available all around them. How populations pay the evolutionary price of traversing from one adaptive peak to the next is at the centre of much of the theory of population genetics. On page 345 of this issue, Clarke and McKenzie² report the use of a novel measure of the disruption of development to assess the response of a

population to the incorporation of an evolutionary novelty.

Much of the controversy about the nature of evolutionary change arises from the difficulty of measuring the cost of passing from one state of adaptation to another. Quite frequently, populations must respond to an environmental challenge by taking advantage of a new mutation with a major influence on fitness. The complexity of the process of development means that such mutations are likely to influence many aspects of the phenotype. Their pleiotropic effects may therefore mean that the population must undergo a period of reduced fitness during the process of adaptation, and in some cases that developmental constraints will limit its ability to evolve³. R. A. Fisher⁴ pointed out nearly 70 years ago that there will then be strong selection for modifier genes that minimize the harmful effects of a new mutation on the developing phenotype while maximizing those that allow adaptation to the new environment. In laboratory mice, for example, it is possible to

reduce the developmental disruption caused by a new morphological mutant by breeding from individuals who show relatively little abnormality. However, the mutant can be restored with its full virulence by crossing it into another genetic background so that amelioration of its effects has arisen from a restructuring of the genomes rather than as a result of change in the allele itself⁵.

The ascent of a new adaptive peak can also be seen in mutants of cultivated plants such as sweet peas and cotton in which older varieties have less disadvantageous side-effects than do those that have only recently arisen⁶. Until now, however, this process has not been observed in natural populations.

Because both sides of a bilaterally symmetrical animal are a product of the same genome, random deviations between left and right indicate how accurately that genome is able to control the process of development. Fluctuating asymmetry — which is to be distinguished from directional asymmetry such as the universal difference in position of left and right human testes familiar to most readers of *Nature* — can hence give an insight into the cost of evolving towards a new adaptive peak. It is this asymmetry that is investigated by Clarke and McKenzie in this issue².

The Australian sheep blowfly *Lucilia cuprina* is a major pest, and several attempts have been made to control it with insecticides. Resistance to dieldrin evolved within 2 years of its introduction in 1955, and this insecticide was then abandoned and replaced with diazinon. Diazinon resistance appeared by 1967, but this chemical is still in use. Flies resistant to dieldrin show significantly more fluctuating asymmetry in bristle number than do wild-type flies; but individuals who carry the gene conferring diazinon resistance are no more asymmetrical than are flies who have never been exposed to insecticides. Twenty years of evolution have allowed *Lucilia* populations to take advantage of a major mutation by adjusting to its disruptive effects on development. That this does indeed reflect a general adaptive shift rather than a change in the major gene itself is confirmed by the experiments of Clarke and McKenzie, who backcrossed the diazinon resistance gene into a laboratory stock of susceptible flies: the level of fluctuating asymmetry associated with diazinon resistance then exceeds even that of flies resistant to dieldrin.

Asymmetry hence shows the degree to which the population has escaped from the valley of relative unfitness which separates it from a new adaptive peak. Its success in making the transition is manifest in the ability of the diazinon resistance gene to persist in insecticide-free population cages containing susceptible flies from modern wild populations, an ability which disappears when diazinon resistance is

crossed into a genetic background which has never before experienced the mutant⁷. The fitness modifiers responsible for the adaptive shift are not linked to the gene encoding diazonin resistance.

Fluctuating asymmetry has the potential to assess the state of adaptation of a population even when the nature of the selection involved is unknown. In some animals, there is a negative association between fluctuating asymmetry and heterozygosity as measured by gel electrophoresis of proteins⁸. This might reflect selection acting at the protein loci themselves, but is more likely to indicate that individuals with a high level of heterozygosity in the genome as a whole (a small part of which is sampled by electrophoresis) are more developmentally stable and hence better adapted⁹. Inbred, highly homozygous and relatively unfit mice have more fluctuating asymmetry than do those arising from crosses between strains¹⁰. For any highly heritable continuous character controlled by several genes, individuals at the extremes of the distribution of a population are more likely to be homozygous than are those near the mean¹¹, and there is evidence that in some cases at least such individuals have higher levels of fluctuating asymmetry¹². As selection nearly always acts against extreme values of continuous characters, this developmental disruption again indicates the position of an individual on its local peak of adaptation.

Species represent very different sets of adaptations, and it is usually possible to cross the valleys of unfitness which separate them only in the laboratory. In interspecific hybrids of trout there is, as might be expected, considerably more fluctuating asymmetry than in the parental stocks¹³. Habitat disturbance has led to hybridization between two species of sunfish *Enneacanthus* in New Jersey and Con-

nnecticut and once again the hybrids are more asymmetrical than are the parental species¹⁴.

Fluctuating asymmetry hence at least has the potential to provide insight into the state of adaptation of a population and, in principle, could be used to test the extent to which an evolutionary response to a changing environment has been successfully completed. By comparing its value for different characters, fluctuating asymmetry might suggest the strength of selection and the relative importance of traits even when their function is unknown. Comparisons of this kind in fossils and their descendants might even indicate whether evolution has been accompanied by a general increase in developmental stability¹⁵. Work on fluctuating asymmetry has the additional merit of providing information on the genetics of populations quickly and cheaply; and in the present bleak landscape for research in evolutionary biology this may turn out to be its greatest advantage. □

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Computer modelling

Turbulence in fluid dynamics

W. Daniel Hillis

PUT a stick into a flowing stream and if the water is moving slowly the pattern of flow around the stick is smooth and constant. Increase the speed of the flow and an instability develops. Whirlpools begin to form, shedding alternately from one side of the stick then the other as regularly as clockwork. Increase the speed still more and the shedding becomes erratic. Vortices themselves shed vortices and turbulence develops. This progression from laminar flow to vortices to turbulence is no special property of water. The same phenomena occur in fluids as diverse as rarified helium gas, warm treacle and piles of sand. Such systems are a type of cellular automata (Wolfram, S. *Nature* **311**, 419-

424; 1984). At a recent workshop*, researchers met to discuss progress in analysis, implementation and application of these new techniques.

The equations that describe the flow of a fluid, Navier-Stokes equations, have been known for more than a century. Yet except for the most trivial cases they remain unsolvable. In most cases the progression from one stage of the flow to the next remains an empirical observation, unsupported by analysis. In practice, the flow pattern of a fluid around a complex object can be predicted only by numerical methods, that is, by floating-point approxi-

mations of iterative approximations to the solution of discrete approximations of the equations.

Recently, progress in parallel computers has encouraged the development of an entirely different approach to predicting the flow of fluids, based on simulating the interactions of individual particles. Because the qualitative behaviour of the fluid is largely independent of the detailed properties of the particles, the rules of interaction can be chosen in such a way as to make them especially easy to compute. For example, under certain circumstances it is possible to use particles that travel only at discrete velocities on a fixed lattice. Also, the number of particles can be much smaller than in physical reality, as long as the mean free path between collisions is small compared with the size of the features being observed.

The biggest question addressed at the workshop was whether these discrete models give the right results. For the two-dimensional flow of an incompressible fluid at moderate velocities, the answer is certainly yes. One popular model (U. Frisch, Observatoire de Nice; B. Haslacher, Los Alamos National Laboratory; Y. Pomeau, L'Ecole Normale Supérieure) uses particles travelling at unit speeds on a hexagonal lattice. Several participants demonstrated analytically that this model produces the Navier-Stokes equations in the incompressible limit (D. Levermore, Livermore National Laboratory; S. Wolfram, Univ. Illinois; M. Henon, Observatoire de Nice). This in itself is significant because it was by no means obvious that the necessary large-scale isotropy could be achieved in a system with preferred axes of motion. The trick is in constructing systems where the detailed structure of the fine-scale interactions do not show through in the aggregate large-scale behaviour. Only some lattices and interaction rules have this property. The analysis predicts the number of particles that must be averaged to display the modelled behaviour to a given degree of accuracy. For realistic engineering simulations this number can be in the thousands or millions.

It is not yet clear how well the method works for more difficult cases. One measure of difficulty is the Reynolds number. As fluids of different viscosity will exhibit similar behaviour at different scales, it is possible to characterize a flow by the dimensionless ratio of inertial force to viscous force, the Reynolds number. As this number increases, a flow progresses from laminar flow to turbulence. To model accurately high Reynolds numbers, the number of particle interactions must be extremely large. Systems with a few million particles accurately model flows of low Reynolds numbers, but turbulent flows may require systems orders of magnitude larger. This could be large enough

*Modern Approaches to Large Nonlinear Physical Systems Santra Fe, New Mexico, 27-29 October 1986.

to defeat even the fastest computers.

In three dimensions the problems are even worse. As well as the need for vastly larger number of particles, there are no agreed rules of interaction. The difficulty is that there is no three-dimensional equivalent to the hexagonal lattice that produces isotropic behaviour. One possible solution is to go to a compact four-dimensional system where suitable lattices are known to exist (P. Lallemand, L'Ecole Normale Supérieure; Frisch). Another is to alternate between several different lattices on successive time steps (Wolfram).

Perhaps the most convincing demonstrations of the technique are the calculations of measurable physical quantities. Researchers have validated their programs by correctly calculating quadratic velocity profiles of plane Poiseuille flow (G. Zanetti, Univ. Chicago), boundary layer profiles (Lallemand, D. d'Humieres) and vortex-shedding rates (B. Nemnich, Thinking Machines Corp.). The most dramatic demonstrations were the animated videotapes showing clear examples of vortex shedding (see cover photograph) and the propagation of shock waves. A special-purpose parallel computer (A. Clouqueur, L'Ecole Normale Supérieure) at the conference allowed participants to try such experiments in real time.

The critical test will be whether these techniques are more useful than conventional methods. This will depend on two things, accuracy and cost. It seems likely

that at high Reynolds numbers these discrete simulations will not give the same answers as numerical methods. There was heated discussion at the meeting as to which answers would be correct. The possibility was even raised that such calculations may, in some cases, be more accurate than the Navier-Stokes equations themselves, because they are in some senses closer to the particulate physical reality. Such speculations were dismissed as unlikely by most of the participants. The cost question is complicated by the fact that the parallel computers that makes this possible also reduce the cost of traditional methods. Analytical techniques using renormalization methods are also being developed (S. Orszag, V. Yakhot, Princeton Univ.).

In a sense the Navier-Stokes equations are serving as a test bed for this style of discrete computation. There are already preliminary results applying these techniques to plasma flow (D. Montgomery, Dartmouth College; G. Doolen, Los Alamos) and to chemical reactions (G. Searby, Univ. Marseilles). Other potential areas of application include stress-strain analysis and the simulation of self-gravitating systems. Whether or not these new methods turn out to be superior, they are likely to offer some stimulating competition to traditional techniques. □

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Cosmology

A new twist for cosmic strings

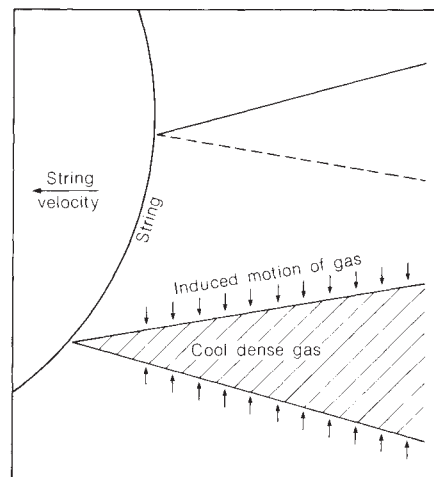
Craig Hogan

Cosmic strings are simple objects that arise in an elegant way from fundamental theory, which is perhaps why theorists have lavished so much attention on them as possible causes of galaxy formation and clustering even though there is no direct evidence for their existence. Unfortunately, if strings actually did exist in the real Universe, then one might expect a series of inelegant complex responses characteristic of many real astrophysical systems. M.J. Rees (*Mon. Not. R. astr. Soc.* **222**, 27-32P; 1986) has recently outlined a situation in which such nonlinear responses could swamp the effects expected in a more naive theory, and even thereby generate structure on a much larger scale.

The Rees model is based on the observation that as a string sweeps through matter it leaves a 'wake' (which I described in a News and Views article, see *Nature* **310**, 365; 1984). The matter on each side of the sheet swept out behind a cosmic string is given a kinematically induced velocity of several kilometres per second (for typical parameters) towards

the sheet (see figure). For collisionless matter, this produces a wedged-shaped region (with the string at the leading edge) in which matter is streaming in two opposite directions. But for normal collisional gas, such as exists everywhere in the early Universe, counter-streaming is impossible, and instead matter crashes into a wedge-shaped shock front where it is slowed down and its energy thermalized. The heated gas then cools, so that the string leaves a thin wake of cool dense gas.

Rees points out that although these wakes comprise only a tiny fraction ($\sim 10^{-6}$) of the matter, they can be coherent on the scale of the strings. Because strings move close to the speed of light, the coherence scale of the string network is generally the scale of the Universe itself (the 'horizon size', or the expansion time divided by the speed of light), and the effect is to produce thin cooled sheets of gas stretching across the visible Universe. Because of the cosmic expansion, this scale was smaller in the past than now, but nevertheless the first such sheets to form after the Universe



The wake produced by a cosmic string. Counter-streaming gas behind the moving string shocks and cools to form a thin dense sheet. The sheet thickness is greatly exaggerated here; in actuality the opening angle of the wedge is of the order of the ratio of gas velocity (a few km s^{-1}) to string velocity (a few hundred thousand km s^{-1}).

recombined at 10^{-3} its present size (redshift of 10^3) would now have a size of the order of 100 Mpc, comparable with the scale of giant sheet-like structures sometimes claimed to be observed in the galaxy distribution and much larger than the typical, ~ 20 -Mpc clustering scale of galaxies.

String-induced sheets of cool gas are, however, too thin to make galaxies directly, and in any case the mass involved in the wakes is much too small to be directly responsible for the bulk of the observed clustering. But the sheets may be unstable, and the indirect effects of inducing local gravitational collapse could be very significant. The gas could, for example, form sheets of stars, whose luminosity, metal enrichment or explosive energy could trigger or modify the formation of galaxies; independently of the details of how this occurred, it would happen with a coherence scale of ~ 100 Mpc. The scenario is most unique in this respect, insofar as correlations on a 100-Mpc scale are introduced without actually moving matter this far, indeed they are introduced at a time (redshift ~ 200) when the bulk of the gas has not collapsed into structures on any scale and is still uniformly distributed.

Because so many of the pieces of the galaxy-formation puzzle remain to be fitted together, it is not yet clear whether this scheme will find a place in the final assembled picture of the origin and evolution of cosmic structures. On the other hand it is certainly an interesting new embellishment of the cosmic string model and one that is likely to be incorporated into future unified accounts of string-induced galaxy formation. □

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Peptide function

Is chromogranin a prohormone?

Lee E. Eiden

IN THE 4 December issue of *Nature*¹, K. Tatemoto and co-workers report the sequence of a newly discovered putative hormone, pancreastatin, which as its name implies inhibits insulin (and somatostatin) secretion from the endocrine pancreas. There is a striking structural similarity between pancreastatin and part of bovine chromogranin A, whose sequence was recently reported in *Nature*² by myself and collaborators, and independently in the *EMBO Journal* by Benedum *et al.*³. This similarity suggests that chromogranin A is a prohormone precursor for pancreastatin or a pancreastatin-like peptide. If so, Tatemoto and co-workers will have contributed decisively to the elucidation of a least one of the physiological functions of chromogranin A. Its role has eluded neuroendocrinologists since its discovery 20 years ago^{4,7}.

Chromogranin A is the chief representative of a family of acidic glycoproteins that are abundant throughout the neuroendocrine system⁸⁻¹¹. Chromogranin A is about 10 per cent of the protein weight of catecholamine-secreting chromaffin cells of the adrenal medulla and is found, often with related proteins variously called chromogranins B and C and secretogranins I and II (ref. 12), in the brain, pituitary, retina, thymus, parathyroid, sympathetic nervous system, enteric nervous system, endocrine gut and endocrine pancreas⁸⁻¹¹. It is from the last-named tissue that Tatemoto *et al.* isolated the 49-amino acid 'pancreastatin' whose structure and biological activity, together with the recently determined structure^{2,3} of chromogranin A, may finally give a clue to the function of chromogranin A.

Pancreastatin, a potent inhibitor of insulin secretion from the isolated rat pancreas, is quite similar to residues 243–294 of bovine chromogranin A (residues 261–312 of prechromogranin A): 71 per cent of the amino-acid residues of the two peptides are identical (see figure). The similarity is even greater (80 per cent, with one gap) in the carboxy-terminal 41 amino acids of pancreastatin. This is significant in that all the biological activity of pancreastatin seems to be localized in the carboxy-terminal 17 amino acids of the peptide¹. The next six nucleotides of bovine chromogranin A messenger RNA encode the dipeptide Gly–Lys, a potential processing signal for proteolytic cleavage and carboxy-terminal amidation, consistent with the isolation of pancreastatin as an amidated species. At the amino terminus, chromogranin A contains a lysine residue, which may be the amino-

terminal processing signal for the formation of pancreastatin from a chromogranin A-like precursor in the rat.

There is chromogranin, as well as pancreastatin, immunoreactivity in the pancreas of several species, and both polypeptides are present in the brain^{11,15}, consistent with a precursor-product relationship between chromogranin A and pancreastatin. Whether or not pancreastatin is generated from chromogranin A or a chromogranin A-like precursor must await the sequencing of the porcine chromogranin A complementary DNA, now under way, or the demonstration of the analogous pancreastatin in the bovine pancreas.

GWPQ--AP--AMDGAGKTGAEEAQPPPEGKGAREHSRQEETATAGAPQGLFRG
KETQRAAPGWPEdgagkmgAEeAKPPEGKGEWAHSRQEETE~MARAPQVLFRRGGK

* * * * * * * * * * * * *

Top sequence, porcine pancreastatin; bottom sequence, bovine chromogranin A 243–296 (pre-chromogranin A 261–314)². Single-letter code for amino acids. Stars, identical residues; dashes, gaps introduced into either sequence to maximize alignment; hash, conservative substitution.

Alternatively, the pancreastatin-like peptide potentially encoded by chromogranin A and pancreastatin itself could be related members of a larger, hitherto unidentified family of peptide hormones analogous to the secretin 'superfamily' of gut hormones¹³. Benedum *et al.*³ have characterized a second bovine adrenomedullary messenger RNA encoding a chromogranin A identical in size and with 98 per cent of the peptide and 94 per cent of the nucleic acid sequences identical to the chromogranin messenger RNA characterized by us². Eberwine *et al.*¹⁴ report that sequences hybridizing to a pancreastatin-complementary oligonucleotide probe may be found in as many as five separate genes in the rat. These observations suggest that chromogranin A and pancreastatin are members of a peptide hormone superfamily. For the moment, both possibilities seem equally likely. Structural analysis of porcine chromogranin A or bovine pancreastatin is needed to distinguish between them.

Several additional questions must be answered to clarify the relationship between pancreastatin and chromogranin A. Does chromogranin A indeed generate a pancreastatin-like peptide in adrenal medulla, endocrine pancreas or any other tissue? Is native unprocessed chromogranin A itself a pancreastatin? These questions are crucial because the ubiquity of chromogranin A in the neuroendocrine system would suggest that the role of pancreastatin or pancreastatin-like molecules is not limited to the pancreas. In fact plasma levels of chromogranin A are sufficiently high¹⁶ (about 3 nM) to stimulate pancreastatin receptors even if only a

small percentage of plasma chromogranin was processed to a pancreastatin-like molecule, or if native chromogranin A had only a fraction of the pancreastatin potency of the peptide characterized by Tatemoto *et al.* It is clearly necessary to establish that the molecule isolated by Tatemoto *et al.* is indeed the substance found at beta-cell and D-cell receptors *in vivo*. It will be of special interest to determine if amidation is necessary for biological activity, because amidation of other biologically active peptides such as enkephalins seems to occur differentially in central and autonomic nervous tissue, and this differential post-translational modification may be relevant to different, as yet undiscovered hormonal functions of these molecules. In addition, there is precedent for cleavage at single basic amino-acid residues within the precursors of other biologically active secretory peptides¹⁷ and thus pancreastatin residues

1-49, 14-49, 27-49, 31-49 and 36-49 (or chromogranin A 244-294, 248-294, 260-294, 267-294, 272-294, 279-294 and even 287-294) could be the actual biologically active peptide(s) produced by and secreted from any endocrine tissue.

In any event, the demonstration of biological activity for a chromogranin A-derived molecule would be a partial reward for the considerable efforts so far made to characterize and understand this ubiquitous but puzzling molecule. Further information validating or disproving a biosynthetic and/or genetic relationship between pancreastatin and chromogranin A, and linking chromogranin A to other endocrine functions, should accrue quickly.

Two of the authors of ref. 3 independently report the sequence similarity between chromogranin A and pancreastatin on page 305 of this issue¹⁸. □

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Nucleosynthesis

Gamma rays from galactic ^{26}Al

Giovanni F. Bignami

THE main evidence that heavy atomic nuclei are still being fashioned by stars throughout our Galaxy was the observation of the 1.809-MeV gamma-ray line arising from the decay of ^{26}Al , an isotope with a half-life of only a million years, by the High Energy Astronomy Observatory (HEAO-3)¹ and Solar Maximum Mission (SMM)² satellites a few years ago. But the recent data consisting of just 80 photons detected by the MPE (Max-Planck-Institut für Extraterrestrische Physik) balloon Compton telescope³, which has a much better angular resolution than the satellite instruments, seem to place the ^{26}Al close to the galactic nucleus (see figure) rather than spread diffusely over the whole galactic disk, as had been concluded from the HEAO-3 and SMM observations. Webber, Schönfelder and Diehl have also now proposed⁴ an elegant explanation of the ^{26}Al , if it is centralized in the Galaxy, by coupling it with the already well-established 0.511-MeV positron annihilation line also seen originating from the centre; so the 80 photons on which the MPE result is based appear to have at least weakened the evidence for 'uniform' nucleosynthesis in the Galaxy.

The MPE balloon instrument, which images the gamma rays by Compton scattering, produces maps with a resolution of around 10° FWHM within its 60° field of view, and has an energy resolution of 11 per cent at 1.8 MeV. It was flown in October 1982 from a base in Brazil for southern sky exploration (it also observed gamma rays from the radio galaxy Cen A), and during the last hours of the flight had the galactic centre in its field of view. The data analysis of a double Compton instrument is never easy, involving such subtleties as 'distributions of angular residuals of event circles' for arrival direction determination and the 'mirror method' for background subtraction; but finally the authors³ have derived both a spectrum and a map of the observed radiation. In the spectrum they claim there is evidence, to within 4.5 standard deviations, for a 1.8-MeV line fully consistent with that expected from ^{26}Al . From the map (see figure), the authors conclude that the shape of the image is consistent with what would be expected from a point source, and that the maximum probability for the position of the source is very near the galactic centre.

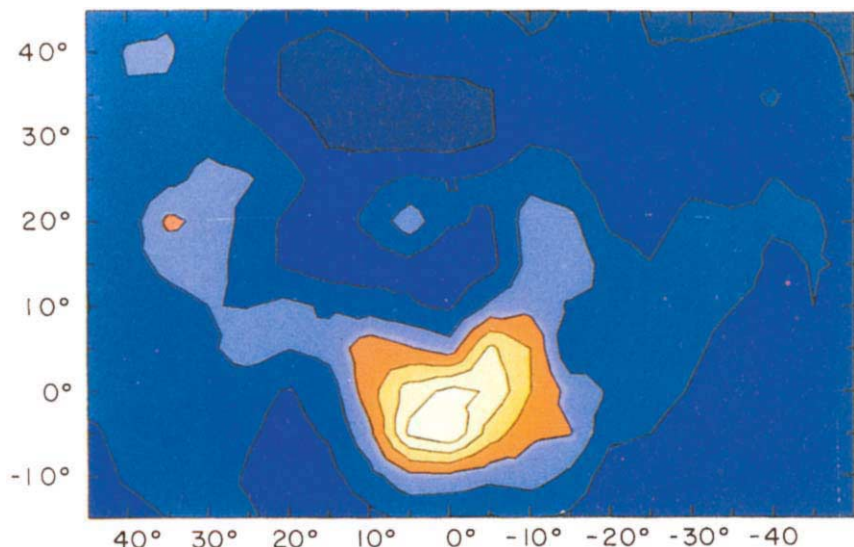
However, an unresolved source, as seen by their³ moderate angular resolution, could still be extended by up to several degrees, and as such may not be truly concentrated at the very centre of our Galaxy. The MPE map is nevertheless in

significant (if not sharp) disagreement with the HEAO-3 and SMM data, where, with angular resolutions of 40° and 130° respectively, it is claimed that the 1.809-MeV line comes from the galactic disk with a distribution similar to that of the diffuse high-energy gamma rays from the interstellar medium observed with the COS-B satellite.

What does this mean for galactic nucleosynthesis? Detection of the 1.809-MeV

should produce a 1.16-MeV line much stronger than the 1.809-MeV ^{26}Al line; but this has never been observed. Theoretically, simple novae, with 150 million kelvin in their thermonuclear explosions, seem to fare somewhat better, at least from the point of view of yield: with 3×10^{-8} solar masses of ^{26}Al per explosion, and with a nova rate of more than 40 per year, at least a good fraction (1–2 solar masses) of the total observed aluminium could be accounted for, again in a million years.

Normal, non-explosive stellar evolution, the basis for the synthesis of the stuff we are made of, is also important for ^{26}Al production and subsequent injection into the interstellar medium: Wolf-Rayet and rapidly mass-losing stars are the obvious



Map (in galactic coordinates) of the central regions of the Galaxy in ^{26}Al 1.8-MeV gamma rays. Ordinate, galactic longitude; abscissa, galactic latitude.

photon of the ^{26}Al decay chain is proof that active nucleosynthesis has been occurring for at least the past million years (the ^{26}Al half-life), a relatively short timescale when compared with that of galactic evolution. Starting from the pioneering work of 'B²HF' (ref. 5), nucleosynthesis was placed as much in normal stellar evolution as in explosive events such as novae and supernovae. The latter events seemed particularly attractive both because they easily meet the energy requirements for the production of heavier elements (because nuclear transformations proceed in the shock-wave ejection of the core and envelope material) and because of their high temperature and densities.

Recent work by Clayton⁶, however, shows that the total amount of ^{26}Al from the gamma-ray observations (4 solar masses) is too much by a factor of around 30 for production in the 30,000 or so supernovae, the maximum number that can have happened in our Galaxy in one isotope half-life. Also, ^{26}Al from supernovae should be associated with ^{44}Ti , which

candidates in this context. Through the work of Norgaard⁷, and now of Cameron⁸, red giants have also been shown to produce significant ^{26}Al in the hydrogen burning at the base of the outer convection zone, whence it can be expelled into interstellar space. Finally, it is clear that all the processes mentioned, from supernovae to asymptotic giant branch stars, could be at work at the same time, and the 1.8-MeV data could reflect a mixture of disk populations dispersing ^{26}Al in the interstellar medium. But in all such calculations quantities are imprecise, and great uncertainties exist in the flux of gamma rays expected.

If, on the other hand, the contention by von Ballmoos *et al.*¹ that a strong concentration exists towards the centre is correct, most such models would face many difficulties. True, one can play with different disk distributions of novae and find acceptable fits to the MPE data, but it also seems appropriate to think of something quite different. There might be, for example, a high concentration of supernova events in the galactic centre, perhaps in multiple or giant form. In this context,

both Hayakawa⁹ and Hillebrandt *et al.*¹⁰ have considered giant explosive events in the centre. As mentioned above, Webber *et al.*² have proposed an elegant solution, based on the idea of associating the only two gamma-ray astronomy line measurements from that general region of the sky. According to these authors, the previously observed 0.511-MeV line could come from the annihilation of positrons emitted in the ²⁶Al-²⁶Mg decay, a process that is immediately followed by the emission of the 1.809-MeV photon bringing the ²⁶Mg to its ground state. In this model a significant part at least of the observed ²⁶Al would not be created from continuous diffuse nucleosynthesis in the disk, but rather from the special environment in the galactic centre.

What all these results clearly call for are better data, providing good spectral resolution as well as serious imaging for the gamma-ray sky. Cooled germanium detectors already provide a spectral resolution approaching 0.1 per cent around 1 MeV, as shown at least in part by the HEAO-3 experiments, and are currently improving. Imaging in gamma-ray astronomy is trickier, because of the physics of high-energy photon detection. True imaging (at the arc minute level, a minimum requirement for astronomical work) is possible with coded masks or 'multiple pinhole' collimators, but this has so far never been coupled with the high-resolution spectroscopy possible with germanium detectors. A mission combining the two, and with good sensitivity in a wide range, is currently being considered by the European Space Agency under the name of GRASP (Gamma-Ray Astronomy with Spectroscopy and Positioning) for launch on whatever carrier may be available in the early-to-mid 1990s.

Looking beyond nucleosynthesis, much new astronomical data would be revealed to a mission looking for the first time at gamma-ray sources with the same resolution as the historical imaging proportional counter on the Einstein Observatory: from the active galactic nucleus gamma-ray luminosity function, to new redshift measurements in the 0.511-MeV line, to a wealth of galactic problems such as accurate mapping of the ²⁶Al line. □

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Earth sciences

Composition of the inner core

Raymond Jeanloz

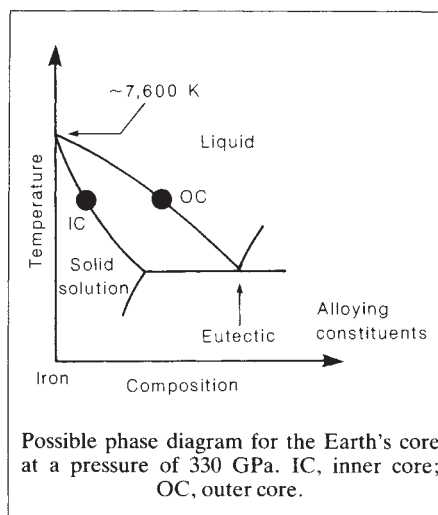
THE inner core makes up only 0.7 per cent of the volume of the Earth. Despite its small size, this region is very important to our understanding of the planetary interior. In particular, current estimates of the temperature at the centre of the Earth; of the energy sources required to sustain the geomagnetic field; and of the core heat that is available to drive convection in the overlying mantle all rely on models of the chemical and physical state of the inner core. Thus, A. Jephcoat and P. Olson's reanalysis elsewhere in this issue (*Nature* **325**, 332-335; 1987) of the geophysical constraints on the composition of the inner core is of broad interest.

By comparing the seismologically determined density of the inner core with the latest measurements of iron and iron-sulphide densities at elevated pressures, Jephcoat and Olson conclude that some alloying constituent must be present in the solid inner core, as well as in the liquid outer core. This result is somewhat tentative because seismological constraints on the inner-core density are less certain than for other regions of the Earth. Also, the combined effects of temperature and pressure on the experimental measurements are still imperfectly known. Nevertheless, Jephcoat and Olson's conclusion is supported by the most recent shock-wave study on iron reported by J. M. Brown and R. G. McQueen (*J. geophys. Res.* **91**, 7485-7494; 1986). Although Brown and McQueen suggest that the inner core is pure iron, their analysis in fact shows that both the density and bulk sound velocity of iron are about 5 per cent higher than those of the inner core, when compared at the same pressures and temperatures (see Fig. 6 of their paper).

A possible phase diagram for the core alloy at the pressure of the boundary between the inner and outer core is shown in the figure. The coexisting solid (inner core) and liquid (outer core) compositions are shown in accord with Jephcoat and Olson's estimate that about half the alloying of iron is indicated by the inner-core density relative to that of the outer core. If this analysis is correct, it will be possible to combine further refinements of the seismological densities with high-pressure measurements (now in progress) on the melting relations of iron-rich alloys to identify the temperature at which the inner core and outer core compositions coexist. Because the inner core is sufficiently small to be essentially isothermal, such a determination of the temperature at the inner-core boundary is equivalent to measuring the temperature at the centre

of the Earth.

The compositional separation between the inner core and outer core is also of interest to those modelling the dynamo that produces the geomagnetic field. One of the leading models for driving this dynamo is a compositionally induced convection of the outer core, whereby denser (more iron-rich) crystals freeze out of the less dense (more alloyed) outer-core liquid. The crystals thus sink towards the inner core, and in doing so induce the liquid motions that sustain the magnetic field. According to Jephcoat and Olson,



however, the compositional and hence density difference between inner- and outer-core materials is substantially less than previously thought. In the light of their results, they suggest that the dynamo is sustained mainly by the alternative energy source of radioactive decay. Models of radioactive heating lead to the prediction of considerably higher fluxes from the core into the mantle than for the compositional dynamo. Hence, Jephcoat and Olson favour core heating as a significant source of energy for mantle convection and the resultant motion of crustal plates observed at the surface.

Regardless of whether one fully accepts Jephcoat and Olson's conclusions, their timing is appropriate not only because it is the 50th anniversary of the discovery of the inner core, but also because very recent advances in seismology and in high-pressure experimentation have led to a resurgence of studies on the deepest region of our planet (see, for example, the December issue of *Geophysical Research Letters*). □

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Enzyme catalysis

Antibodies with some bite

David E. Hansen

THE spectacular specificities and rate enhancements of enzymes are without equal among all known catalysts. It is no wonder then that the design of new enzymes has long been a goal of biochemists. Now two independent groups, one led by Alfonso Tramontano and Richard Lerner of the Scripps Clinic and Research Foundation¹, and the other by Peter Schultz of the University of California at Berkeley², have taken steps towards this goal by demonstrating that antibodies can have catalytic activity.

These accomplishments stem from almost a century of research on enzyme catalysis that began with the legendary chemist Emil Fischer. In 1902, Fischer³ stated in his Nobel Prize lecture

The examination of synthetic glucosides has shown that the action of the enzymes depends to a large extent on the geometrical structure of the molecule to be attacked, that the two must match like a lock and key. . . . To equal Nature here, the same means have to be applied, and I therefore foresee the day when physiological chemistry will not only make extensive use of natural enzymes as agents, but when it will also prepare synthetic [enzymes] for its purposes.

Since then there have been revolutionary advances in our understanding of enzyme catalysis and of protein structure, but it is a measure of the immense complexity of enzymes that a completely synthetic system has yet to be prepared. Present thinking on the basis of enzyme catalysis began in the 1940s when Linus Pauling suggested that Fischer's Lock and key analogy must be modified: an enzyme active site should not match the substrate, it should rather be complementary to the activated complex (or transition state) of the reaction being catalysed. Much recent work on enzyme mechanisms has supported Pauling's view, and one may convincingly argue that most of the principles of enzyme catalysis are now well understood (even if we do not necessarily see how these principles are manifested).

Although a concomitant understanding of protein folding and structure has not so far been achieved, a strategy for generating new enzymes that depends on an understanding of catalysis, but not on the ability to design polypeptides that will fold properly, was first alluded to by Pauling⁴ in 1948

An enzyme has a structure closely similar to that found for antibodies, but with one important difference. Namely that the surface configuration of the enzyme is not so closely complementary to its specific substrate as is that of an antibody to its antigen, but instead is complementary to the activated complex.

William Jencks⁵, more directly, stated in 1969

If complementarity between active site and transition state contributes significantly to enzyme catalysis, it should be possible to synthesize an enzyme by constructing a binding site. One way to do this is to prepare an antibody to a haptenic group which resembles the transition state of a given reaction. The combining sites of such antibodies should be complementary to the transition state and should cause an acceleration by forcing bound substrate to resemble transition state.

With the potential for more than 10^{12} different antibody molecules, one may speculate that every antibody catalyst imaginable is obtainable from the existing repertoire of antibodies (just as every antibody needed to bind all antigens seems to be accessible). The challenge is therefore in selecting an antibody with the desired catalytic function from this huge repertoire. However, as pointed out by Jencks, if in a stable molecule one could model the transition state for a reaction, such a selection process might be at hand.

Although several early attempts to isolate antibodies with catalytic activity failed⁶⁻⁸, the transition state analogue strategy has now succeeded. Both the Scripps group and the Berkeley group used tetrahedral phosphorus compounds to mimic the presumed transition state in the hydrolysis of a carboxylate ester. Tramontano *et al.* generated monoclonal antibodies against four phosphonate esters and demonstrated that some of these antibodies have catalytic activity (these workers distressingly call these catalytic antibodies 'abzymes'). The values of the first-order rate constant k_{cat} at pH 8 for two ester substrates with the monoclonal antibody 6D4 are 1.62 min^{-1} and 0.48 min^{-1} . The corresponding K_m values, the concentrations of substrate required for half-maximal velocity, are $1.90 \mu\text{M}$ and $0.62 \mu\text{M}$.

Schultz *et al.* tested the mouse monoclonal antibody MOPC167, which is known to bind nitrophenyl phosphorylcholine, as a catalyst for the hydrolysis of an analogous carbamate and measured a k_{cat} of 0.4 min^{-1} and a K_m of $208 \mu\text{M}$ at pH 7. These rates correspond to accelerations of approximately 1,000-fold over the uncatalysed rates, but are approximately 5,000-fold slower than, for example, the rate for a good ester substrate of α -chymotrypsin.

Both research groups note that their antibodies have many of the same characteristics as enzymes: each shows good substrate specificity, exhibits saturation

kinetics and is subject to competitive inhibition. Although the detailed chemical mechanisms of these catalytic antibodies have yet to be determined, it is reasonable to suppose that the ester or carbamate is strained towards a tetrahedral geometry on binding, thus facilitating the attack of the hydroxide ion. Furthermore, the Scripps group has already found that their antibody can act via both nucleophilic and general base catalysis, depending on the substrate used.

The fact that this approach succeeded at all gives great hope to those attempting to isolate even more efficient antibody catalysts by this and by other strategies. In addition, it may be possible to modify existing catalytic antibodies. Because the crystal structures of monoclonal antibodies containing their bound haptens can be determined — and in fact such a crystal structure had already been solved for the antibody used by the Berkeley group — site-directed mutagenesis can be intelligently used to change particular amino acids so as to generate enhanced specificity or catalytic function. In addition, both groups are planning to attach co-factors and metal ions to the antibodies in attempts to increase catalytic efficiency further. One immediate goal is the creation of sequence-specific proteases, which would have many important biochemical applications. Overall, the possibilities appear almost limitless, and as Schultz's group concludes, "these approaches may enable us to tailor-make catalysts for use as tools in biology, chemical synthesis and medicine".

Given the fact that antibody molecules can act as catalysts, it will now be possible to address several theoretical questions about enzyme catalysis. For example, enzymes are flexible molecules that may assume different conformations during a catalytic cycle, but the contribution of these motions to catalysis remains uncertain. Antibodies, on the other hand, although not static molecules, seem to have only one important functional conformation. It will be of great interest to see if antibodies with catalytic efficiencies equal to those of enzymes can be obtained, or if, ultimately, fundamental differences between antibodies and enzymes will limit this approach. □

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Data in graphs and tables

SIR—The ambiguities inherent in the presentation of data in graphs and tables in the ways discussed by Ferreira (*Nature* **324**, 215–216; 1986) may be eliminated by applying the multiplier term to the physical quantity, not to the unit. Thus, to use the example given by Ferreira, the axis would be labelled:

$10^{-3} \times \text{NADH concentration } (\mu\text{M})$

or

$10^{-3} \times \text{NADH concentration}/\mu\text{M}$

with scale marks of 2, 4 and 6 to represent NADH concentrations of 2,000, 4,000 and 6,000 μM respectively.

In this presentation the axis labels (or table headings) imply:

$10^{-3} \times \text{NADH concentration}/\mu\text{M} = 2$

which rearranges to:

$\text{NADH concentration} = 2,000 \mu\text{M}$

This method is the one recommended to authors by the *Biochemical Journal*.

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Chromogranin A and pancreastatin

SIR—Tatemoto *et al.*¹ recently reported the sequence and biological activity of a pancreatic peptide, which they named pancreastatin because it was found to inhibit glucose-induced insulin release from the pancreas. The authors suggested that pancreastatin "may belong to a hitherto unknown peptide family". We have noticed a rather striking resemblance between the amino-acid sequence of pancreastatin and that of chromogranin A deduced from cDNA sequences reported independently by us² and Iacangelo *et al.*³

Of the bovine chromogranin A sequence between residues 251 and 294, 70 per cent of the amino acids are identical to those of the 49 amino acids of porcine pancreastatin. In addition, the presence of glycine at position 295 in chromogranin A is consistent with the C-terminal amide structure observed in pancreastatin. The most striking difference between the two sequences is the lack of amino acids 5–8 of pancreastatin between residues 254 and 255 of chromogranin A.

We believe that the sequence resemblance between the bovine chromogranin A segment and porcine pancreastatin is too great to be coincidental. In line with previous sequence data suggesting that chromogranin A may be a precursor of regulatory peptides^{2,3}, one possible explanation of the observation is that pancreastatin is produced from chromogranin A by limited proteolysis. Such a precursor-product relationship between chromogranin A and pancreastatin is attractive because the endocrine pancreas contains chromogranin A^{4,6}. But as chromogranin

A is a secretory protein occurring in a wide variety of peptidergic endocrine cells^{4,6} and neurons⁷, we find it unlikely that its only function is that of a precursor to pancreastatin.

Lee Eiden, an author of ref. 2, independently reports the sequence similarity between chromogranin A and pancreastatin elsewhere in the News and Views section of this issue⁸.

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Origin of hominid bipedalism

SIR—Sinclair *et al.*¹ suggest that hominid bipedalism evolved as a response to selection pressure for long-distance travel to scavenge from migrating ungulate herds. I suggest that simple temporal considerations of hominid evolution render a causal relationship between scavenging and emergence of bipedalism as highly implausible.

An examination of functionally relevant traits of the lower limb, such as shortened distance between sacroiliac and hip joints, carrying angle of the femur, feet with convergent big toes, short toes and arches, indicate that habitual erect posture and bipedalism had evolved in *Australopithecus* by 3.5 million years ago². Throughout the evolutionary history of *Australopithecus* the dental, gnathic and cranial material suggests a high degree of vegetarianism for these early hominids. There is neither morphological nor archaeological evidence that the total dietary repertoire of *Australopithecus* included a larger meat-eating component than that of chimpanzees and baboons. The earliest clear evidence for scavenging, butchering practices and meat-eating is associated with early *Homo* starting around 2 million years ago³.

The observation that more or less regular meat-eating postdates the emergence of bipedalism by considerably more than one million years falsifies the hypothesis of bipedalism having evolved in response to long-distance travel in pursuit of migrating ungulate herds. In my view the most convincing idea on the origin of hominid erect posture and bipedalism rests on Jolly's feeding hypothesis, in its refined version⁴. This proposes that the hominid locomotor pattern evolved in early *Australopithecus* as an adaptation to gathering and eating the young leaves, seeds, and pods of the ubiquitous thorn scrub of

the African plain. These foods grow upon bushes that are too spiny and limber to climb, and too high to pick from a pronograde position. In addition, the earliest hominid, *Australopithecus afarensis*, had relatively short hindlimbs⁵, another argument against long-distance travel during that phase of hominid evolution.

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SIR—Sinclair *et al.*¹ believe that human bipedalism arose in scavenging hominid ancestors that had to carry their children while following migrating savanna ungulates but this seems highly improbable.

There was no empty niche of migrating scavengers to be occupied by hominid ancestors. Not only vultures, but also canid, felid and hyaenid carnivores were much better preadapted for such a niche. They possessed sharp beaks or long canine teeth and did not need to carry stones for cutting carcasses. Moreover, the bipedal way of locomotion—whether fast or slow—is inefficient and costly^{2,3}.

Another argument against the migrating hypothesis in particular and the savannah theory of human evolution in general is that it is highly unlikely that our hominid ancestors ever lived in the savannas. Man is the opposite of a savanna inhabitant. Humans lack sun-reflecting fur⁴ but have thermo-insulative subcutaneous fat layers, which are never seen in savanna mammals. We have a water- and sodium-wasting cooling system of abundant sweat glands, totally unfit for a dry environment⁵. Our maximal urine concentration is much too low for a savanna-dwelling mammal⁶. We need much more water than other primates, and have to drink more often than savanna inhabitants, yet we cannot drink large quantities at a time^{7,8}. The fossils of our hominid ancestors or relatives are always found in water-rich environments.

It is difficult to understand why most anthropologists keep believing in the savanna theory (possibly because it goes back to Charles Darwin), or why so many anthropologists keep trying to seek the most improbable reasons for bipedalism, while they should know that there are much better explanations^{9–11}.

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Divergent genetic codes

SIR—A recent News and Views article by Grivell¹, commenting on a paper by Hanyu *et al.*², and an earlier commentary by Fox³ both addressed evidence for divergence from the universality of the genetic code in some ciliated protozoa. These organisms apparently use UAA (ochre) and UAG (amber), which are normally stop codons, to encode glutamine in polypeptides. Hanyu *et al.* provide evidence for the existence of two unusual glutamine tRNAs in *Tetrahymena thermophila*, one of which recognizes both UAA and UAG codons in messenger RNAs, whereas the other recognizes only UAG codons. In considering the evolution of tRNAs which can use UAA and UAG as sense codons in polypeptides, Hanyu *et al.* suggest “the stop codons UAA and UAG were rarely used during prociliate evolution and that weak suppressor tRNAs existed for these codons very early. Spontaneous point mutations creating UAA and UAG codons within protein genes would then not have been lethal for these prociliates. . . . Once those early suppressor tRNAs acquired anticodons for perfect UAA and UAG recognition, respectively, the use of these codons as amino-acid codons became fixed”. We would like to draw attention to supporting evidence in the form of weak amber and ochre suppressor tRNAs in yeast that are normally changed with glutamine.

We have shown that the yeast *Saccharomyces cerevisiae* contains two normal tRNA^{Gln} genes, one bearing the anticodon UUG and the other CUG^{4,5}. When present on multicopy plasmids, the tRNA^{Gln}_{CAA} gene effects weak suppression of two different ochre mutations⁴, whereas the tRNA^{Gln}_{CAG} gene suppresses a number of different amber mutations⁵. In both cases, codon–anticodon interactions between the nonsense codons and the tRNAs requires U–G mispairing at the first rather than the third codon position which may occur by weak first position wobble in organisms that use the conventional genetic code. More recently, by use of a strategy that involves deleting the single genomic copy of the essential yeast tRNA^{Gln}_{CAG} gene and rescuing the potential lethality resulting from the deletion by transforming cells with a multicopy plasmid carrying the tRNA^{Gln}_{CAA} gene, we have shown that

suppression of UAG codons can even result from a single copy of a tRNA^{Gln}_{CAG} gene, thus strengthening the physiological relevance of this phenomenon as an evolutionary mechanism for divergence of the genetic code (W.A.W., I Edelman, M.R. Culbertson and E.C.F., unpublished data).

Based on established rules for codon–anticodon pairing we hypothesize that the evolutionary progenitors of the ciliated protozoa under consideration might possess tRNAs with the conventional anticodons UUG and CUG, which can decode the universal glutamine codons CAA and CAG. Random mutations in regions of these tRNAs outside of the anticodons could be readily tolerated during evolution, as there is no compelling *a priori* reason for their counter-selection. Given the precedent for limited readthrough of UAA and UAG codons in yeast^{4,5}, we argue that altered glutamine tRNAs with enhanced stability for first codon position wobble might in fact be strongly favoured in organisms in which the stop codons UAA and UAG had entered the coding region of essential genes by random genetic drift. Selection for these tRNAs could be facilitated by other mutational changes which further enhance the stability of first codon position wobble, for example alterations in ribosome binding and modification of bases in and/or outside the anticodon. Such evolutionary selection could eventually result in highly efficient suppression of these ‘stop’ codons, resulting in their retention in the coding region of genes of ciliated protozoa. This model is consistent with the fact that *Tetrahymena thermophila* contains a family of cognate tRNAs, some of which can decode CAA and/or UAA codons and others of which can decode CAG and/or UAG codons².

Grivell also speculates on codon recognition by glutamine tRNA species in organisms which branched off the main line of eukaryotic evolution before or about the same time as the ciliates. He notes that *Dictyostelium discoideum* and *Trypanosoma brucei* (both of which branched off before the ciliates) show no deviation from the standard genetic code. According to some studies, the yeast *S. cerevisiae* may have branched just after the ciliates, with a much closer interval between the ciliates and yeast than that between the ciliates and *D. discoideum* or *T. brucei*⁶. Our observation¹ that *S. cerevisiae* shows the vestiges of “an intrinsic property of a primitive or error-prone code” supports the hypothesis that misreading of nonsense codons by normal glutamine tRNAs (which has been preserved in yeast) may have preceded the use of UAA and UAG as sense codons for glutamine.

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Mystery object identified?

SIR—Artefacts closely resembling the mystery object recently encountered by Wolstenholme *et al.* (*Nature* **323**, 300; 1986) in a chromosome preparation are routinely fabricated in electronics laboratories by means of positive-resist electron-beam lithography. During the fabrication of certain semiconductor devices, a layer of poly[methyl methacrylate] (called PMMA) about 0.5 to 1 µm thick is deposited on a silicon wafer. The PMMA layer is irradiated in a given pattern with an electron beam having a spot size as low as 0.01 µm in diameter. The irradiated areas, being of reduced molecular weight, are then dissolved in a solvent that attacks the lower-molecular-weight areas. This leaves a pattern of holes in the PMMA layer; such patterns have resolutions of 0.1 µm or better. Normally the remaining PMMA pattern is dissolved by a solvent during subsequent processing steps. If in the course of experimentation the wafer is discarded before the PMMA pattern is dissolved, it is possible for the pattern to peel off the wafer and enter the environment as a dust particle.

It does not strain credulity to consider the mystery object as a fragment of a PMMA pattern that has contaminated the chromosome preparation. A regular grid of squares of the size suggested by the photograph of Wolstenholme *et al.* is a useful test pattern for experiments in semiconductor device fabrication techniques; the ‘black’ squares in the photograph are probably air bubbles.

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WOLSTENHOLME REPLIES—In addition to the published ‘explanations’ of our ‘mystery object’, others have been sent directly to us. The majority of these suggest an extraterrestrial origin for the object but we prefer an explanation closer to home.

The various biological ‘grids’ that have been drawn to our attention — some of which are very beautiful — are never as regular as ours. We therefore prefer to believe that the object is man-made, although exactly what it is and how it arrived in our preparations we are still at a loss to understand. We agree that the black squares are probably air bubbles.

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Are we stuck with sex?

John Maynard Smith

Origins of Sex: Three Billion Years of Genetic Recombination. By Lynn Margulis and Dorion Sagan. *Yale University Press: 1986. Pp. 258. \$35, £30.*

THIS book differs in background and viewpoint from other recent studies of the evolution of sex. It draws heavily on a knowledge of the structures and life histories of primitive eukaryotes — highly relevant topics that have been largely ignored up to now. The authors fear that the novelty of their approach will meet with resistance from the members of an “entrenched, restricting thought community”. They may well be right; for example, they reject the question of why sexual organisms are not replaced by reproductively superior asexual ones as “not a valid scientific question”. Since I have spent much of the past 25 years thinking about just this question, I cannot be expected to approach their book with unalloyed enthusiasm.

Let me start, therefore, by saying that I am in complete agreement with Margulis and Sagan on two fundamental points. First, sex, in the sense of the bringing together in a single individual of genetic material from two different ancestors, is not necessary for reproduction. Second, many of the enzymes that mediate genetic recombination in animals and plants originated in prokaryotes as DNA repair enzymes.

Before voicing my criticisms, I will first summarize the argument. The authors see the evolution of sex as occurring in a series of stages: I think that they could be right about some of these, and wrong about others. In brief, enzymes for cutting, splicing and recombining DNA evolved in early prokaryotes because of their role in repairing damage caused by ultraviolet light. The processes of transformation, transduction and conjugation, whereby DNA from different sources comes together in a single cell, evolved because the repair of double-strand damage requires the presence of an homologous copy of the damaged gene. Eukaryotes evolved from symbiotic communities of prokaryotes. In particular, the structures which today move cells about (“undulipodia”, a word the authors use to distinguish the cilia of eukaryotes from the structurally quite different flagella of bacteria), and the structures that move chro-

mosomes about inside cells (mitotic spindles) are derived from a spirochaete-like symbiont. The fusion of similar eukaryotic cells (misis) probably evolved from cannibalism, because it paid to retain the DNA of an ingested relative, rather than to digest it. Meiosis then evolved to restore

their single-celled ancestors. This is that cells able to produce an undulipodium cannot undergo mitosis, presumably because the same organizing centre is required for both processes. They see cellular differentiation as a “numbers game”, analogous to ecological succession: the state of a cell is determined by the copy number of different genetic entities (chromosomes, genes, organelle DNA) within them, just as the state of an ecosystem is determined by the numbers of different species. That is, differentiation is caused by differential amplification. To restart development, however, this state of differentiation must be returned to an initial

state. This is achieved when a haploid cell is produced by meiosis. Higher organisms, therefore, must retain meiosis because it is a necessary part of any life cycle involving cell differentiation. Sex is maintained because higher organisms have no option, and not because of any advantage it confers in generating variability or accelerating evolution.

So much for the thesis. How much of it can we accept? I have said already that I accept that recombination enzymes originated as repair enzymes. However, the account of prokaryotes is muddled and full of inaccuracies. For example, the diagram of DNA replication shows new bases being incorporated in the wrong place; it is not true, as stated, that it is the recipient cell in conjugation that develops pili; and the account of a bacterial cross that produces a recombinant able to survive in an environment lethal to both parents is wrong (although the principle is correct). More seriously, the authors have not thought things through carefully enough. For example, they point out that induction of some phages is caused by ultraviolet light, and suggest that the process evolved because

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Aphid birth — meiosis is no requirement for development.

haploidy. A regular haplo-diploid cycle, with alternate miosis and meiosis, evolved as an adaptation to a cyclical environment, because diploidy was favoured when conditions were harsh, and haploidy when rapid reproduction was possible. There is some evidence that haploids multiply faster than diploids in favourable conditions: the advantage of diploidy in harsh conditions could result from gene complementation.

Multicellularity, and cellular differentiation, were forced on a particular eukaryotic lineage because of a peculiarity present in existing animal and plant cells which the authors see as being present in

DNA wrapped up in a protein coat is more resistant to ultraviolet than DNA in a cell. I do not know what the evidence for greater resistance is, but it may be correct. However, the process looks to me more like a device by genes in the phage to ensure their own survival when the host cell is in trouble, than one that ensures the survival of bacterial genes. The mechanisms of evolution in prokaryotes are fascinating, and different from those in eukaryotes, but they require a rather more careful analysis than the authors seem to have been willing to give them.

The best part of the book is that concerned with the origin and early evolution

of the eukaryotes. Margulis and Sagan define the "protocists" as those eukaryotes that are neither animals, plants or fungi. I cannot help wondering how the cladists will react to such a definition — I am on the authors' side, although I wish they had chosen a term more compatible with the principles of English pronunciation. They persuade me that these organisms provide a rich and rewarding field of study, with which I ought to be more familiar. I have for some time been convinced by the arguments for the symbiotic origin of the eukaryotes. The structural similarities between cilia and spindles argue strongly for a common ancestry. However, the significance of the RNA associated with these structures seems still to be obscure. I was fascinated to learn that "a tightly linked cluster of genes... may be carried on the kinetosomal RNA", but disappointed to find that the reference for this was to S.K. Dutcher, personal communication. To discover the role of this RNA would seem to be a high priority for students of protist genetics.

I find it plausible that cannibalism should have played a role in the origin of sex. The idea that the cycle of mixis and meiosis is an adaptation to a cyclical environment is attractive, and, so far as I know, new.

I part company with the authors when they come to discuss the evolution of animals and plants. I do not see how multicellularity could have been forced on a lineage by the fact that a single cell could not both divide and have a cilium. What, after all, could the single-celled ancestor have been doing? Further, I do not think that differentiation can be explained by differential gene amplification. The process certainly occurs, but is probably unimportant compared with differential gene expression.

The essential weakness, however, lies in the claim that meiosis is needed to reset the developmental process. Note, however, that the claim could be true even if development depends on differential gene expression and not on differential amplification: thus meiosis might be needed to reset the state of gene expression, perhaps by altering patterns of methylation. The point is that many higher organisms can develop from a single cell that is not the product of meiosis. In most higher plants, many single somatic cells can give rise to a new individual. In animals and plants, there are many parthenogenetic varieties that do not undergo meiosis: this is true, for example, of the many triploid parthenogens, and of diploids such as the permanently asexual strains of cladocerans and aphids. If meiosis were a requirement for development, such organisms could not exist.

In case it should be thought that I am misrepresenting the authors, let me give a short quotation:

Animals and plants that show no mixis, which is to say organisms that have lost the capacity to form offspring from different parents in sexual unions (such as apomictic or self-fertilizing plants), nevertheless retain meiosis. We suggest they do this to maintain differentiation. Outcrossing has never been shown to confer a definitive advantage on organisms. Leguminous plants are surely successful yet they generally are self-fertilized. Dandelions have bypassed mixis entirely, yet their "success" is undisputed [p.180].

Leaving aside the fact that most leguminous plants are not self-fertilizing, this is an odd passage. Most dandelions are triploid apomicts, and do not undergo meiosis. It is certainly true that in some large taxa (for example, mammals and gymnosperms) no ameiotic organisms are known. In these, the argument that sex is retained because meiosis is a precondition for development may be correct. But in many taxa, ameiotic parthenogens are not

uncommon, although their taxonomic distribution suggests that they are short-lived in evolutionary time. For these taxa, the argument is manifestly false. The authors write "Although plants and occasionally animals can escape mixis, they cannot go for long without meiosis". This gets it exactly the wrong way round. Plants, and many animals, can escape meiosis, but they cannot go for long, in evolutionary time, without mixis.

This is a stimulating and irritating book. It has convinced me that important clues to the origins of sex are to be found in the study of primitive eukaryotes. But it is confusingly written and needlessly repetitive, and its main thesis is disproved by the widespread occurrence of ameiotic parthenogenesis among plants and animals. □

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Picture physics

J. H. Mulvey

The Particle Explosion. By Frank Close, Michael Marten and Christine Sutton. Oxford University Press:1987. Pp.239. £15. To be published in the United States on 9 April by OUP, New York, \$35.

AN ENIGMATIC lady, perhaps from the East, clad to the fingertips in black, gazes down at a green surface patterned in yellow lines and curls. She might be divining the fates of Caesars from the entrails of a sacrificial animal. In fact she is interpreting the motions of sub-atomic particles revealed as lines of bubbles in one of the ingenious inventions of the physicists who study the fundamental constituents of matter. Her photograph faces the opening page of this beautifully illustrated history of, and guide to, particle physics — a mix of seeming science fiction and abstract art. The authors are clearly aiming at the coffee tables of everyone whose curiosity can be awakened about the physical make-up of the world and how we have come to understand so much about it.

The Particle Explosion thus takes on the

challenge of those who say the subject is too esoteric, and also dares comparison with the many popular picture-filled books on natural history and astronomy, topics which are more familiar by being, at least superficially, part of their readers' experience. At a scale of 10^{-15} m and below we encounter a new world of particles and forces; the constituent quarks and the 'colour-force' which imprisons them within the proton and neutron, and the 'weak-force' which can transform one sort of quark into another releasing, as it does, the radiations of radioactivity. One of the most dramatic developments of recent years is the link now seen between the laws of physics which rule in this micro-world and the evolution of the Universe in the very early moments of the Big Bang.

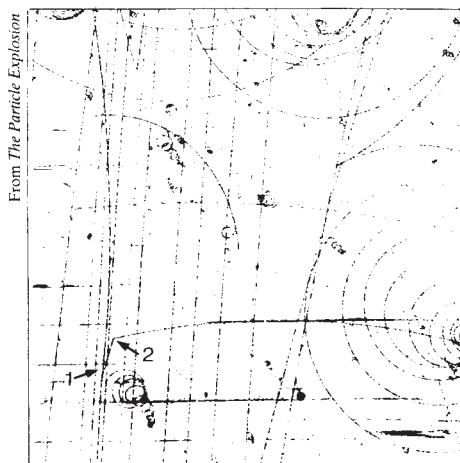
The pictures are stunning, many of them cleverly chosen to train the reader as an amateur observer of the traces left, like foot-prints in the snow, by particles as they flash through the apparatus arrayed to detect them. The story is told easily and straightforwardly (no mathematics), is enlivened by snapshots of the principal personalities and holds the attention well — as judged by a Christmas sample of readers including a social worker and two (non-scientist) university students. The book is



Public exposure — a French cartoon from the turn of the century, reflecting the public's fascination with X-rays which had just been discovered by Röntgen.

divided into easily digestible chapters, almost complete in themselves, which deal, alternately, with the physics of the particles and with the increasingly large and complex tools, particle accelerators and detector assemblies, necessary to study them.

Good though the writing is, the pictures make the book. Where did the almost surrealist photograph of a direction-sign for a fall-out shelter, standing desolate in the Utah desert, come from? Blips on an oscilloscope trace signalled the first detection of the 'undetectable' neutrino by Reines and Cowan; kinks in cloud-chamber tracks revealed to Rochester and Butler the existence of new out-of-the-ordinary,



From *The Particle Explosion*

Historic track — the first picture to show the production (1) and decay (2) of an omega-minus particle, taken at the Brookhaven National Laboratory in the early 1960s. Discovery of the omega-minus led quickly to the quark theory of matter.

hence 'strange', forms of matter. The prize pictures, justifying the adjective 'explosive', must however be those of the most violent man-made collisions — recreating conditions which have not existed since the Universe was 10^{-9} s old — in which W and Z bosons were transiently observed, so confirming the union of the electromagnetic and weak forces. Missing, however, is the lone electron straggling through the dark vault of the Gargamelle bubble-chamber at CERN which was the first direct sign of this major synthesis. The surprise of its absence measures the completeness of this pictorial feast.

Many people will enjoy this attractive, reasonably priced volume and its story of the technical achievements which have brought the denizens of the sub-atomic world into view. Novel concepts such as quarks, leptons and gauge bosons will eventually become, like the atom is today, part of the commonly accepted picture of the natural world. Books such as this will help to speed the process. □

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Policing the paper chase

Stephen Lock

An Insider's Guide for Medical Authors & Editors. By Peter Morgan. ISI Press, Philadelphia/STM Distribution, Ashford, Middlesex: 1986. Pp. 111. Hbk \$23.95, £20.50; pbk \$14.95, £13.

LIKE it or not, the scientific editor's role has changed. Once concerned mostly with house style and peer review, and balancing the competing elements in his journal, he has now had other preoccupations thrust upon him: duplicate publication; piracy, plagiarism and forgery; and hidden commercial interests. Examples of transgressions occur in many issues of *Nature* or *Science*, but let me cite two recent problems encountered personally.

In the first, one author was found to be submitting over 20 articles a year to the *British Medical Journal* alone; a literature search showed that he and his team were publishing double that number altogether. (Solution: a tactful check that the data weren't invented, followed by a letter pointing to the heavy costs of manuscript processing — about £50 in our case — and suggesting that weightier papers rather than those based on the least publishable units might be more successful.)

Secondly, a couple of junior investigators asked for advice when a professor in another department added his name to three articles reporting work which he had had nothing to do with. (Solution: follow the American practice of involving the dean and the overall head of the division.)

Clearly we need a guide to the new "journalology", a term some editors in the United States are now using with only just a hint of tongues in their cheeks, and Peter Morgan, editor of the *Canadian Medical Association Journal*, has set out to give us the first of its kind. His *Insider's Guide* does not neglect the traditional advice and detailed methods for better scientific writing: to base original articles on the IMRAD structure (introduction, methods, results and discussion) while making them readable by following the style books, such as Fowler or Strunk, and cutting out abbreviations and jargon. But, with his epidemiological background, Morgan stresses that, whereas in the early twentieth century William Osler could make his name with over 1,400 articles, most of them single-case reports, today's emphasis has shifted to the testing of hypotheses. Inevitably many of these articles are flawed, and no amount of stylistic tinkering can repair them because the studies they report were misconceived from the start. They have committed the seven deadly sins — insufficient informa-

tion, biased or inadequate samples, confounding factors, vague endpoints, straying from the hypotheses and poor control of numbers.

Hence much of the thrust of Morgan's book is on numeracy — the presentation in tables and figures of data that examine the hypotheses and analysis of the data for statistical significance. Scientists in other disciplines may be surprised at the need for such emphasis in medicine, but in a subject that still contains as much art as science many teachers still do not accept this need and journals abound with examples of poorly conducted trials. If, then, in the 1960s and 1970s one of the main preoccupations of medical editors was with the ethics of human and animal experimentation, that in the 1980s and 1990s is likely to be with statistical rigour. For this, they will find Morgan a trustworthy guide, though I remain unconvinced that his use of folksy anecdotes, such as Drs Relso, Nojarg and Terse discussing randomized clinical trials in a court house, is any better for explaining the type II ("false-negative") error than a straight account.

Medicine, however, can claim one success: it is the first discipline in which journal editors have collaborated to formulate proposals for uniform style. Widely accepted, these Vancouver guidelines must have saved authors (and their secretaries) hundreds of hours. The International Committee has produced guidelines on reference style, duplicate publication and criteria for authorship, while it is discussing proposals for retraction of fraud or erroneous comment. Would that others would copy. For without being snide, it has to be said that our two principal general science journals do their readers a disservice by failing to give the full bibliographical details in the references. On average, only six or so more lines are needed per article and the gain in rigour, accuracy and usefulness is immense.

It is no accident that many of the scandals about shady work (whether forged or plagiarized) have occurred in medicine. The stakes are high — a single article in the *New England Journal of Medicine* can have immediate repercussions on the New York Stock Exchange, for there are fortunes to be made with a better drug for hypertension or rheumatoid arthritis. And, while appointments committees continue to assign merit on the weight of articles in a curriculum vitae, rather than to read a selected few for quality, the publish or perish syndrome is unlikely to go away. Perhaps the often-ridiculed proposal of allowing any scientist to publish only five articles a year (or even 50 in a lifetime) was not such a bad one after all. □

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Me scientist, you chimpanzee

John C. Marshall

Ape Language: From Conditioned Response to Symbol. By E. Sue Savage-Rumbaugh. *Columbia University Press/Oxford University Press: 1986. Pp.433. \$40, £30.*

If I want to communicate effectively with members of a culture radically different from my own, there are two basic options open to me: either I learn their language or they learn mine. When the representatives of a different culture are also members of a different species, exactly the same options arise. Saint Francis (and later Karl von Frisch, Konrad Lorenz and Nico Tinbergen) chose the first route; Sue Savage-Rumbaugh (and earlier Beatrice and Allen Gardner, and David Premack) opted for the second. For sociologists, the fact that Europeans were prepared to adapt themselves to the world of the animals they studied, whilst Anglophones insist that the animals try to do it our way, may point a moral. For the rest of us, the continuing saga of the chimpanzee's ability to communicate in a human-like fashion shows no sign of losing its fascination.

Savage-Rumbaugh places her own contribution to the debate firmly within a neodarwinian context. The great apes are our closest living relatives: "...amino acid sequencing, immunological, and electrophoretic methods of protein comparison between man and chimpanzee all indicate that the average human polypeptide is more than 99 per cent identical to its chimpanzee counterpart". Yet despite this biochemical similarity — "comparable with that found among sibling species who are virtually identical in terms of morphology" — taxonomists continue to place man and chimpanzee in different families. That we should think of ourselves as being so very different from the great apes when they "share our biological heritage to a remarkable degree" seems somewhat perverse to Savage-Rumbaugh. The distinction, she writes, "has no biological basis, but appears to be derived instead from a singular behavioral distinction — the ability of man to speak". This argument is deeply puzzling, both in its equation of biology with the constitution of polypeptides, and in its implication that human language does not rest upon specific biological foundations. It is as if one were to argue that a Picasso and a Matisse must be highly similar because, when the paintings were ground up and centrifuged, they had a near identical spectrum of pigment types. Nonetheless, the evidence of genotypic similarity

suggests to Savage-Rumbaugh that the phenotypic uniqueness of man has been greatly exaggerated. What better way, then, to demonstrate behavioural similarity than to show that the chimpanzee can, in an appropriate environment, acquire at least the rudiments of language skills as they are manifest in all normal human children?

The general drift of such a research programme has been familiar since the earliest days of biological classification. Linnaeus had placed the orang-utang in the human genus, albeit as a species separate from man; and Buffon claimed that the orang-utang was not a man solely because it could not talk. Lord Monboddo, the eighteenth-century Scottish linguist and eccentric, was unconvinced that the orang had been given a fair opportunity to demonstrate its prowess, and he proposed

"As with watching a circus horse walk on its hind legs, I could not escape the feeling that a species ill-adapted to symbolic communication was struggling with an unnatural task."

a simple solution: we should patiently teach an orang-utang to speak. If the animal could learn a language then it was indeed a primitive man. Some 200 years later, the chimpanzee has been substituted for the orang, the research programme has been carried out, and we now know that the answer is nowhere near as simple as Monboddo hoped.

In *Ape Language*, Savage-Rumbaugh makes no attempt to show that her young male chimpanzees, Sherman and Austin, can acquire even a simplified syntax. With the exception of the Gardners, no serious student of ape language now seems convinced that the chimpanzee can make productive use of a system in which *People tickle chimpanzees* means (roughly) the same as *Chimpanzees are tickled by people*, has a different meaning from *Chimpanzees tickle people*, and in which *People by tickled are chimpanzees* doesn't mean very much at all. But a language consists (minimally) of a syntax and a vocabulary. And for Savage-Rumbaugh the dominant question has become: can a chimpanzee demonstrate an appropriate, symbolic use of vocabulary items to communicate information to a human trainer or to a conspecific animal?

The question goes considerably beyond the well-known facts of natural animal signals, and of arbitrary response conditioning. If, severely provoked, I turn red and snarl, a conspecific may back away, but my expression is not the name of my emotional state; likewise, if properly conditioned, I raise my right hand each time the experimenter shows me a yellow circle, my gesture is not the name of the visual stimulus I was presented with. What evidence, then, would we require of a

chimpanzee (or a human child) before we were prepared to concede that the use of a particular overt sign had symbolic significance, that it, in some sense, 'stood for' the concept of, say, *banana*?

The two most impressive demonstrations of symbolic communication that Savage-Rumbaugh reports are these. Austin sees on a television screen a tray of different foods; the screen is too small for him to point and indicate which food he wants. Austin goes to a computer console, and by pressing a key he lights up an arbitrary geometrical shape from a selection of such shapes that (in previous training sessions) had been associated with different foods. Sherman observes this sign, goes to the next room, wherein is found the real food tray. From this tray, he selects the correct food (having, like Austin, been previously trained in the relevant associations) and brings it back to Austin to eat. Both chimpanzees (eventually) became remarkably skilled in this game. Second demonstration: Sherman observes a tray of objects. He then goes to the keyboard and lights up a sign corresponding to one of them. The sign is transmitted by projectors to the trainer, who cannot see the chimpanzee. Sherman then returns to the tray, selects the object whose sign he pressed and gives that object to the trainer. Again, both chimpanzees (eventually) became highly adept.

Savage-Rumbaugh interprets the first experiment as the chimpanzee requesting that a particular food be brought, the second as announcing that a particular object will be brought. Exactly how pleased John (*How To Do Things With Words*) Austin would have been with Austin's performance, we can leave other philosophers to decide. For the moment, these demonstrations remain the best evidence we have for the deployment of an arbitrary symbolic code by any non-human primate. The 433 pages of *Ape Language* do, however, amply document just how difficult it was to get the chimpanzees to play these word-games. As with watching a circus horse walk on its hind legs, I could not escape the feeling that a species ill-adapted to symbolic communication was struggling with an unnatural task. We are still far from the day when an audience of humans is addressed in the terms of Franz Kafka's chimpanzee: "Honoured Members of the Academy! You have done me the honour of inviting me to give your Academy an account of the life I formerly led as an ape". □

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• Two other recent books on ape language — *Gavagai! Or the Future History of the Animal Language Controversy* and *Silent Partners: The Legacy of the Ape Language Experiments* — were reviewed by E. W. Menzel Jr in *Nature* 323, 497 (1986).

Channelling and channelling radiation

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The study of channelling phenomena has developed rapidly since the early 1960s and today channelling has found many applications. The radiation emitted by channelled megaelectronvolt and giga-electronvolt electrons and positrons has been investigated extensively and the possibility of, for example, constructing intense tunable X- and γ -ray sources is being explored. Multi-giga-electronvolt radiation and pair-creation processes in single crystals show similarities with strong-field effects and are of particular interest because of high production rates that persist far beyond the channelling regime.

In the early 1960s, measurements of the range and energy loss of positively charged ions in single crystals revealed directional effects. With incidence close to low index axes or planes the depth penetrated into the target was observed to exceed significantly that obtained in a corresponding amorphous material^{1,2}. Computer simulations showed similar features³. The results were interpreted as an easy passage of ions along open channels in the crystal lattice, a possibility which had been ignored for half a century after its first prediction by Stark⁴ in 1912. The effect was named *channelling*.

In 1965, Lindhard published a comprehensive theoretical study on the 'influence of the crystal lattice on motion of energetic charged particles'⁵. His major emphasis was on the axial case where he introduced the concept of an atomic string—a row of atoms—and showed that the governing of projectile motion by the crystal takes place for a much wider class of trajectories than those just confined to a single open channel. An expression was derived for the angle of incidence characteristic for directional effects to appear. For well-aligned beams an estimate was given for the reduction in yield of close encounter processes, that is, processes that require the projectile to hit right at the centre of target atoms⁶. Experimental verifications of Lindhard's 'string effect' followed promptly⁷ and by now the entire field, still known as channelling, is well established⁸.

Channelling played a decisive role in the early investigations in ion implantation and semiconductor technology. It provided the basis for rapidly expanding fields within both fundamental and applied physics. Today, practical applications of channelling are widespread^{9,10}.

Channelling experiments have been extended into the giga-electronvolt region of incidence energies. Here, steering effects persist as shown, for example, through the bending of relativistic charged particle beams in smoothly bent crystals^{11,12}, an effect currently considered for practical applications. Also light particles have been channelled and electrons, as well as negative pions, have tested the governing of the motion of negatively charged projectiles which appear significantly different from the positive case^{13,14}. At low energies, quantum phenomena specific to electrons and positrons were studied¹⁵.

The prediction in the mid 1970s of the effect of channelling radiation^{16,17} and the subsequent experimental verification^{18,19,22} strongly revived the field of light particle channelling. Here it was shown how the electromagnetic radiation or 'bremsstrahlung' emitted when an electron or a positron is accelerated in an atomic field may be enhanced significantly for a projectile whose motion is governed by the crystal potential^{12,20}. Under suitable conditions line spectra are observable. The line energies are adjustable through the primary projectile energy. For electrons of a few megaelectronvolts channelled along the axes or planes in, for example, silicon single crystals, line energies of a few kiloelectronvolts emerge, for planar positron channelling at giga-electronvolt impact energies pronounced megaelectron-

volt photon peaks show up with enhancements over normal bremsstrahlung by up to a factor of forty. At present, most aspects of channelling radiation are understood and applications are being investigated²⁰. These range from the use of 'channelling spectroscopy' to obtain information on the electronical and phonon structure of crystals to the prospects of constructing intense tunable X- and γ -ray sources.

Lately, the influence of crystal structure on electromagnetic processes at multi-giga-electronvolt impact energies has been the subject of intense studies theoretically as well as experimentally. This holds true for bremsstrahlung and, in particular, for the inverse process of pair production^{21,22}. Here a photon incident along a crystal axis or plane is converted into an electron-positron pair, which may be channelled. At multi-giga-electronvolt energies, a strong enhancement in the production rate is encountered with respect to the one obtained in amorphous media. This enhancement persists far beyond the angle characterizing the population of channelling trajectories.

The string effect

Directional effects in crystals are due to correlation between successive soft collisions with target constituents. When a particle moves along a low index axial direction and passes close to one atom experiencing only a slight deflection, it must also pass close to neighbouring atoms of the same row. This led Lindhard^{5,6} to his introduction of the atomic string characterized by the constant distance, d , of separation between atoms placed on a straight line. Because the effective action radius of a string typically is an order of magnitude smaller than the string interspacing, collisions occur in lowest approximation with one string at a time. According to Bohr²³ the scattering with a single atom needs a quantum mechanical treatment for projectile velocities above the orbital electron velocities. However, Lindhard was able to show that, because of the correlations in the consecutive atomic collisions, a classical orbital picture applies in the description of the interaction between a heavy projectile and the string⁵.

To establish the characteristics of governed motion consider at first a heavy positively charged ion, incident essentially parallel to a string at a non-relativistic energy. As the deflection angles in the encounters with the individual atoms are very small, the path is well approximated by a straight line during each collision. The corresponding momentum transfer Δp is then perpendicular to the direction of motion, which, in turn, almost coincides with the direction $\hat{z}$ of the string, that is,

$$\Delta p \approx -\frac{1}{v} \int_{-\infty}^{\infty} dz \nabla_{\mathbf{r}_{\perp}} V(\mathbf{r}_{\perp}, z) \quad (1)$$

Here $\mathbf{r}_{\perp}$ denotes the coordinate transverse to the string, v is the projectile speed and V denotes the projectile-atom interaction potential. According to (1), the momentum transfer during the

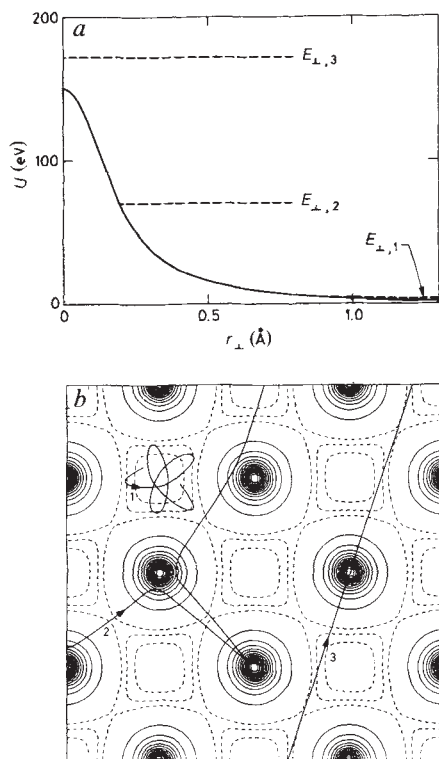


Fig. 1 Transverse motion in the thermally averaged continuum potential of a germanium single crystal oriented along the (100) axial direction and kept at room temperature. *a*, The potential of an isolated string. The regions of transverse space accessible to projectiles moving at three different values of $E_{\perp}$ are indicated by horizontal lines. *b*, The contours of the full crystal potential. The dashed (full-drawn) equipotential curves are interspaced by 1 eV (10 eV), the lowest corresponding to an energy of 1 eV (10 eV) above the potential minimum that pertains to the channel centre. Examples are sketched of trajectories corresponding to each of the transverse energies selected in *a*.

scattering by a single atom may be determined by the integral over a time interval $\Delta t = d/v$ of the force obtained from the potential

$$U(\mathbf{r}_{\perp}) = \frac{1}{d} \int_{-\infty}^{\infty} dz V(\mathbf{r}_{\perp}, z) \quad (2)$$

which corresponds to a charge distribution smeared in the z -direction. Consequently, for motion at small angles to the string, the trajectory of the projectile is governed by the two-dimensional continuum potential (2). It may be noted that the uncertainty in position due to thermal vibrations of the target atoms implies a transverse smearing as well. The resulting 'thermally averaged' continuum string potential remains finite at the string centre, Fig. 1*a*.

In the continuum approximation, there is a complete separation between longitudinal and transverse motion. Because the projectile-string interaction potential (2) is independent of z , the motion parallel to the string is free, that is, it proceeds with a constant momentum, $p_z \equiv \mathbf{p} \cdot \hat{\mathbf{z}}$. As a consequence, the translational energy E_z associated with the longitudinal motion of the projectile of mass M , $E_z = p_z^2/2M$, is conserved. The transverse motion, on the other hand, is governed by U . As the total projectile energy, E , is a constant of motion along with E_z , one is led to the result that the 'transverse energy'

$$E_{\perp} \equiv E - E_z = p_{\perp}^2/2M + U(\mathbf{r}_{\perp}) \quad (3)$$

is a conserved quantity. The region of transverse space accessible to a projectile of given $E_{\perp}$, which according to (3), is composed of the kinetic energy associated with the transverse motion and the potential energy in the interaction with the continuum string,

is defined by the condition $U(\mathbf{r}_{\perp}) \leq E_{\perp}$. Figure 1 illustrates examples of transverse motion for projectiles with different values of $E_{\perp}$.

The special features of the string effect are now easily understood. Consider, for instance, the yield of a close encounter process. In case $E_{\perp}$ is somewhat below the potential maximum $U(0)$, the positively charged particle is kept away from the region near the string of high atomic density, Fig. 1*a*, whereby the yield of the close-encounter process drops off. On the other hand, if the projectile moves at a 'transverse energy' above barrier, $E_{\perp,3}$ in Fig. 1*a*, it hits the target atoms essentially as in an amorphous (random) substance. The critical value of $E_{\perp}$, below which directional effects set in is then $\sim U(0)$. More precisely, as target nuclei may be encountered off their average position $\mathbf{r}_{\perp} = 0$ because of thermal displacements, the slightly lower critical value of $U(\rho)$ should be chosen, where ρ denotes the transverse root-mean-square vibration amplitude. Far away from the string $E_{\perp}$ is purely kinetic and expressible in the form $E_{\perp} = E\theta^2$, where θ denotes the angle of the projectile path relative to the string, cf., (3). The characteristic angle for directional effects is then $\theta \approx \sqrt{U(\rho)/E}$. For any reasonable choice of potential this result approximates Lindhard's critical angle ψ_1 ,

$$\theta \approx \psi_1 \equiv \sqrt{4Z_1 Z_2 e^2 / p v d} \quad (4)$$

where $Z_1 e$, $Z_2 e$ denotes the projectile and target atomic charge, respectively. It may be noted that 'proper channelling', that is, the confinement to a single open channel corresponds to values of $E_{\perp}$ very near the minimum of the crystal continuum potential, Fig. 1. Hence, the corresponding characteristic angle is considerably smaller than ψ_1 .

For incidence near low-index planar directions a similar treatment applies. Because smearing of the charges of the target atoms in this case is performed in two dimensions, the resulting continuum potentials are more shallow than for axes. Correspondingly, directional effects appear for a narrower range of incidence energies with a critical angle of

$$\psi_p = \psi_1 \sqrt{\hat{a}/d} \quad (5)$$

Here $\hat{a} \approx 0.81 \text{ Å} / Z_2^{1/3}$, d^{-2} defines the atomic density in the plane, and, typically, one has $\psi_p \sim \psi_1/3$. In the planar case the condition $E_{\perp} < U(0)$ corresponds to confinement to one channel.

Extension of the theory into the régime of relativistic impact energies where the projectile velocity v approaches the speed of light c is simple indeed: a replacement of E , E_z with their relativistic analogues leaves (3) unaltered except for the introduction of the 'relativistic mass' γM rather than the pure 'rest mass' M into the kinetic energy term,

$$E_{\perp} = p_{\perp}^2/2M\gamma + U(\mathbf{r}_{\perp}) \quad (3')$$

Here the relativistic measure $\gamma \equiv (1 - v^2/c^2)^{-1/2}$ relates to the total energy as $\gamma = (E - U)/Mc^2 \approx E/Mc^2$. The expressions (4)–(5) for the characteristic angles apply directly because the momentum attains the value $p = \gamma M v$.

Examples of experimental verifications are shown in Fig. 2. Part (a) shows the backscattering yield recorded for 480-keV protons incident close to a major axis in a tungsten crystal relative to that measured at incidence far from any low-index crystallographic directions. A dip appears around zero angle with a half width close to ψ_1 , which amounts to a few degrees in the present case. The maximum extinction approaches two orders of magnitude. For parallel incidence the only projectiles that may contribute to close encounter processes are those entering the crystal within a distance ρ from the string. With an atomic density of N the transverse area per string amounts to $(Nd)^{-1}$. Consequently, the normalized minimum yield for close encounters may be given as

$$Y_{\min} = Nd\pi\rho^2 \quad (6)$$

This estimate is in fair agreement with the experimental value. The dashed curve in Fig. 2*a* constitutes the detailed theoretical

prediction. Clearly the overall agreement with the empirical data is very satisfactory.

Figure 2b shows the channelling effect for relativistic protons with an energy of 10 GeV entering a 0.5-mm thick germanium single crystal. Compared with Fig. 2a the energy, which corresponds to $\gamma \approx 10$, has been raised by a factor of $\sim 30,000$! The plot shows the incidence angle of those protons recorded to survive the penetration of the target with a deflection small compared to that encountered on average at random incidence. As revealed by the figure, such projectiles are mainly encountered in channelling domains. The white circle identifies ψ_1 , which here amounts to $\sim 0.01^\circ$. The critical angle is also shown in Fig. 2c, which demonstrates the emission of bremsstrahlung for positron impact near a major axis. We shall return to this topic in later sections.

It is striking how well the fairly simple channelling picture described above applies to all experimental tests performed so far for heavy positively charged projectiles over more than eight orders of magnitude in incidence energy.

Applications

Very soon it was realized that channelling would be an easy and fast method for investigating changes introduced in a crystal upon irradiation with charged particles (implantation). The created damage immediately reflects in an increase of the minimum yield in channelling dips, Fig. 2a. Furthermore, it is possible to locate impurity atoms in the crystal.

Figure 3a shows the principle of foreign atom location in a simple two-dimensional model. A beam of charged particles is incident along the $\langle 01 \rangle$ or $\langle 11 \rangle$ direction. Being channelled in the host crystal, the projectiles are prohibited from traversing the shaded region near the atomic row. Consequently, for impurities placed on substitutional sites (●) channelling dips appear in close encounters with foreign atoms (such encounters may be singled out through observation of, for example, a specific nuclear reaction). For the impurities placed on the positions marked by $\times$ the maximum attenuation observed at incidence along the $\langle 01 \rangle$ direction amounts to 50% whereas no dip in yield appears in the $\langle 11 \rangle$ direction. In fact, for mid-channel impurities a peak is obtained with well channelled particles. The impurity $\square$ yields a full channelling dip in the $\langle 11 \rangle$ direction but none in the $\langle 01 \rangle$. Thus by observing orientation effects along two or more directions, it is often possible to locate the exact site of specific foreign atoms. Fig. 3b, c illustrates this for deuterium implanted in a nickel crystal²⁴—a case of large importance in modern high technology. The experimental results demonstrate the high accuracy of the method.

Among other applications we may mention the use of the crystal blocking technique to measure nuclear lifetimes down to 10^{-17} s (ref. 25). Also the prospects of the use of channelling in slightly bent crystals for steering of gigaelectronvolt beams have received attention. The interest in the latter method rests on the fact that the crystal field with its $\sim 10^{13}$ V m $^{-1}$ exceeds by many orders of magnitude any field produced in the laboratory. Actually, on a single occasion a (cheap) bent crystal has already replaced large (expensive) magnets in a practical beam transport problem.

Electrons and positrons

Already in the early days of channelling, electrons and positrons were introduced as projectiles^{13,14}. Figure 4a shows blocking patterns for these light particles as originating from β -emitting ^{64}Cu atoms implanted on lattice sites in a regular copper single crystal. Blocking is the reverse process of channelling; the fact that, for example, a channelled positively charged particle is prevented from hitting the nucleus of a string atom by the continuum potential barrier implies that the process of capturing a positively charged particle emitted by such an atom into a channelling orbit is prohibited. Correspondingly, in Fig. 4a the yield of emitted positrons shows a pronounced dip when the

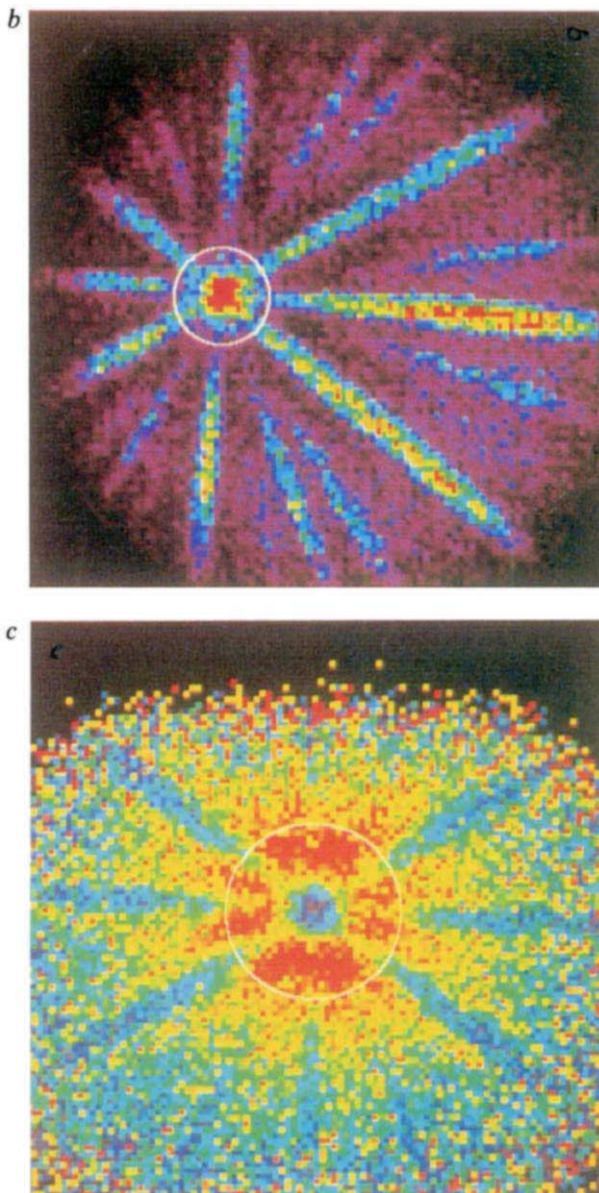
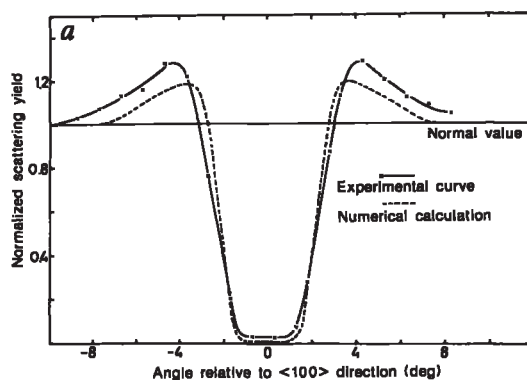


Fig. 2 Experimental verifications of the channelling effect. *a*, Dip in backscattering yield for 480-keV protons incident along a $\langle 100 \rangle$ axial direction in tungsten. *b*, Small-angle scattering, $\theta \leq \psi_1$, for 10-GeV/c protons transmitted through a germanium single crystal in the $\langle 110 \rangle$ direction. Red colours correspond to high intensities, blue (purple) to low. The axis and major planes are clearly visible. *c*, Channelling radiation emitted by 4.8-GeV positrons in a 0.2-mm thick $\langle 110 \rangle$ germanium single crystal. The incident angle is plotted for those projectiles that have caused the emission of photons with energies of 10–40 MeV.

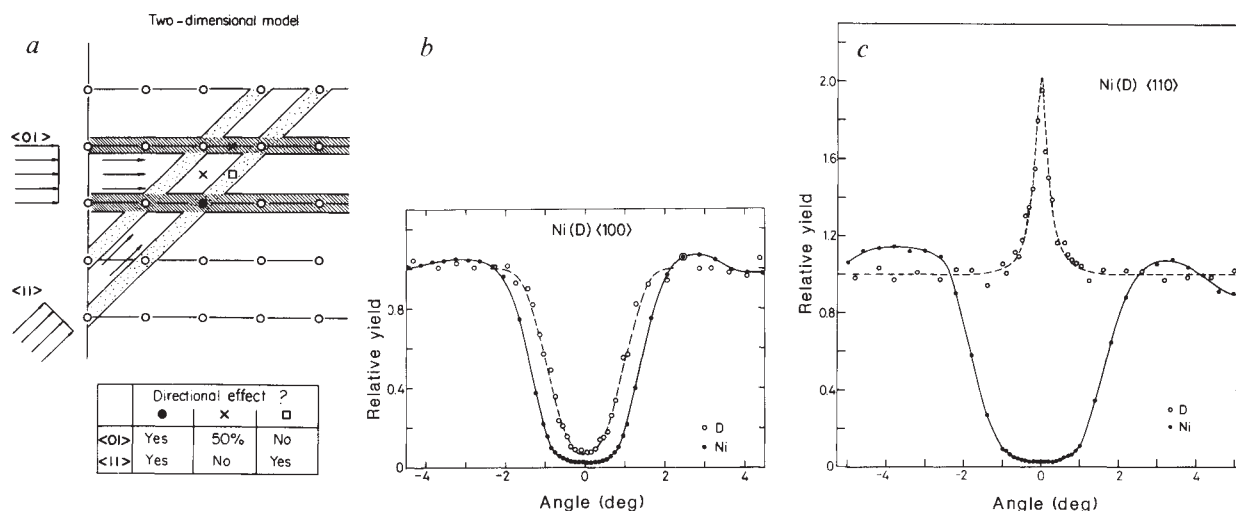


Fig. 3 Foreign atom location. *a*, Principle. *b*–*c*, Normalized channelling yields recorded for deuterium (impurity) and nickel (host) in two different directions. The dashed curves are the theoretical results obtained for the deuterium yield when all impurities are assumed to be at 'octahedral' positions, that is, in the centre of the face-centred cubic nickel structure.

direction of observation is aligned with a crystal axis. On the other hand, the electrons, being negatively charged, see an attractive continuum potential. They tend to focus around atomic positions and, hence, the yield for aligned cases shows a peak.

The above axial dips and peaks yielded for positrons and electrons were in fair agreement with Lindhard's classical theory. Figure 4*b* reveals this feature for the positive case. Here is shown a comparison between scattering yields for external positron and proton beams for the same value of the product pv , compare with (4). The half width of the dip is clearly the same for the two types of projectiles.

The interpretation on purely classical grounds of the electron-positron data, which were recorded at incidence energies of 10^2 – 10^3 keV, met strong criticism, especially from the electron-diffraction community. Furthermore, in his original investigation of the applicability of classical orbital pictures in collisions with atomic strings, Lindhard arrives at the condition that the projectile mass (actually the relativistic mass) be large compared with the electron (rest) mass. Could classical pictures ever make sense for electron-positron channelling?

The classical description of channelling is approached when the discrete spectrum of states of transverse motion allowed by quantum mechanics becomes dense and hence approximates the continuous classical spectrum. An estimate of the number ν of bound states may be obtained as the number of cells of volume h^2 , Planck's constant squared, in the available $(\mathbf{r}_\perp, \mathbf{p}_\perp)$ -phase space specific to a string and corresponding to $E_\perp < \max\{U(\mathbf{r}_\perp)\}$. In the planar case the cell volume equals h . For negative particle axial and planar channelling this procedure leads to the estimates^{15,20}

$$\begin{aligned} \nu_s &\approx \frac{\gamma M 2\tilde{A}}{m} \frac{1}{d} Z_2^{1/3} \sim \frac{\gamma M}{m} \\ \nu_p &\approx \sqrt{\frac{\gamma M 2\tilde{A}}{m}} \sqrt{Nd_p^3} \sim \sqrt{\frac{\gamma M}{m}} \end{aligned} \quad (7)$$

Here m denotes the electron mass and d_p the planar interspacing, which amounts typically to a few Ångströms. The order of magnitude is indicated to the far right. Clearly, for heavy projectiles, $M \gg m$, high numbers are encountered in accordance with Lindhard's analysis.

Also, a classical description is justified for electrons and positrons with impact energies in the gigaelectronvolt region where $\gamma \gg 10^4$. At lower energies, for example, in the kiloelec-

tronvolt and megaelectronvolt region, more care is needed. In the case of axial positron channelling, where states do not appear localized, Fig. 1*b*, the number of bound states essentially always remains high. Consequently, the application of a classical picture in the interpretation of the positron data of Fig. 4 seems justified. However, for electrons, as well as planar channelled positrons, the number of bound states may be low. At a few megaelectronvolts, for instance, electrons may show only a single planar channelling state and less than ten axial ones.

There are cases of practical interest where the quantal structure is essential. Indeed, this is so when the radiation from channelled megaelectronvolt electrons is considered. Quantum mechanically, the wave equation governing the transverse motion is obtained by 'quantizing' (3'). As in the familiar case of atomic spectroscopy, the stationary states of transverse motion, compare with Fig. 5*a*, are found as eigenstates of the emerging Schrödinger-like equation^{15,20}.

Channelling radiation and bremsstrahlung

An electron channelled originally in a higher energy state of Fig. 5*a* may liberate part of its total energy and de-excite to a lower state through the emission of a photon. This phenomenon is known as channelling radiation.

Channelling radiation is associated with governed motion. It is intimately related to the process of 'bremsstrahlung' where a freely moving charged particle impinges on a single (isolated) atom and radiates. Here, photon emission is a result of the acceleration in the atomic field. Classically, the instantaneously radiated power is proportional to the square of the acceleration, a . Consequently, with $a \propto M^{-1}$, only electrons and positrons are of practical interest as projectiles. At relativistic energies, photons emerge inside a narrow cone of opening angle $1/\gamma$ around the direction of projectile motion. The spectrum is independent of the sign of the charge of the projectile and extends up to the primary energy with the emission probability decreasing proportional to the inverse of the quantum energy, $d\sigma/d\hbar\omega \propto 1/\hbar\omega$. For atoms distributed at random in an amorphous medium the bremsstrahlung emitted in the individual encounters adds incoherently.

In case the atom is situated in the regular lattice of a single crystal, interferences may set up between the radiation originating at the various scattering centres. For an electron or a positron that may be assumed to move freely through the crystal the resulting 'coherent bremsstrahlung' has been known for decades^{26,12} with its first prediction dating back to the 1930s²⁷.

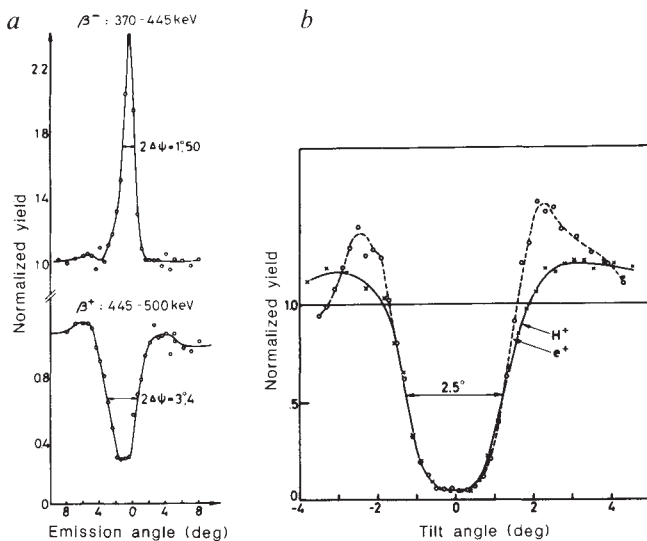


Fig. 4 Electron-positron channelling. *a*, Blocking patterns in a copper single crystal observed along the $\langle 110 \rangle$ axial direction. *b*, Dips in Rutherford scattering yields measured for $\langle 110 \rangle$ axial channelling in a gold single crystal for 1-MeV positrons and 0.670-MeV protons.

The relation between photon energy and incidence angle θ relative to a major crystallographic direction that corresponds to constructive interference may be established in a simple picture by requiring the separation of the wavefronts originating from photon emission in successive collisions with atoms belonging to neighbouring rows or planes interspaced by a distance δ , $\Delta l = (c/v - 1)\delta/\theta$, to equal an entire number of wavelengths, that is, the relation

$$\hbar\omega = 4\pi n\gamma^2 (\hbar c/\delta)\theta, \quad n = 1, 2, 3, \dots \quad (8)$$

is obtained for forward emission when the condition $\gamma \gg 1$ is fulfilled. Still, electrons and positrons give rise to identical spectra.

In channelling radiation, coherence is built in already through the steering of the projectile in its smooth trajectory originating in the large number of correlated soft scattering events with target atoms. Because of this 'cooperation' of target constituents, channelling radiation is in general enhanced over normal (incoherent) bremsstrahlung. As channelling trajectories for electrons are qualitatively different from those of positrons, the radiation spectra are no longer similar.

Megaelectronvolt impact energies

For channelled megaelectronvolt electrons, where the transverse motion is quantized and only a few discrete values of $E_{\perp}$ are allowed, Fig. 5*a*, channelling radiation is emitted in quantum jumps from a higher to a lower lying state. The relation between the shift in transverse energy and the emission angle and energy may be established through requirement of energy and longitudinal momentum conservation.

Alternatively, the emission process is often considered in the so-called rest frame moving through the crystal along the axis or plane in question at the constant longitudinal velocity of the electron. In this frame the motion is non-relativistic and purely transverse and photon emission appears as in the familiar case of atomic spectroscopy, the only difference being the reduction to two dimensions or, for planes, even one. In the rest frame the photon energy evidently equals the quantum jump, $\hbar\omega^R = -\Delta E_{\perp}^R$. However, as the crystal is now rushing by at speed $-v$ it appears Lorentz-contracted, $d^R = d/\gamma$ and, as a result, continuum potentials are magnified by a factor of γ , compare with (2). Also the kinetic energy term in the transverse wave equation is enhanced by a factor of γ in the rest system because the

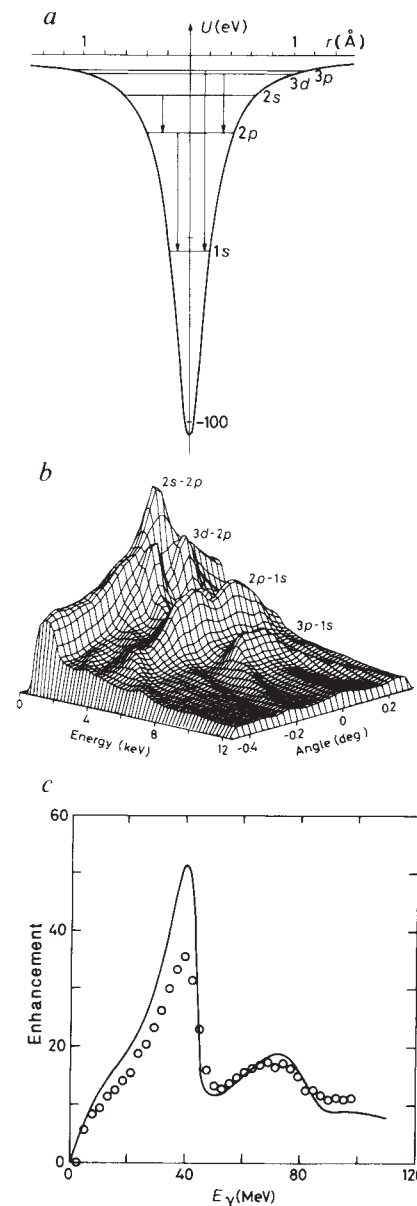


Fig. 5 Channelling radiation. *a*, Discrete spectrum of allowed transverse energy levels for 4 MeV electrons channelled along the $\langle 111 \rangle$ axis of a silicon single crystal at room temperature. The states are identified through their spectroscopic quantum numbers. Possible radiative (dipole) transitions are indicated. *b*, Photon spectra recorded experimentally for a variety of incidence angles of the primary electron near the crystal axis. The conditions are the same as in *a*. Four lines are identified. *c*, Enhancement of channelling radiation over incoherent bremsstrahlung for 6.7-GeV positrons incident parallel to a $\{110\}$ plane in a 0.1-mm thick silicon single crystal. The solid line represents theoretical values.

relativistic mass here equals the rest mass. Hence, as $\mathbf{r}_{\perp}$, $\mathbf{p}_{\perp}$ are unaffected by the transformation, we obtain $E_{\perp}^R = \gamma E_{\perp}$ and thereby $\hbar\omega^R = \gamma(-\Delta E_{\perp})$. A Doppler transformation back to the laboratory frame leads to the final result

$$\hbar\omega = 2\gamma^2(-\Delta E_{\perp})/1 + \gamma^2 \theta_{\gamma}^2 \quad (9)$$

where again $\gamma \gg 1$ has been assumed. The quantity θ_{γ} denotes the emission angle relative to the z -axis. It is worthwhile noting that the factor γ^2 shifts the energy difference $\Delta E_{\perp}$, which is typically in the 10^1 -eV region, compare with Fig. 5*a*, into kiloelectronvolt photon energies. In the laboratory frame, the major part of this energy given off by the projectile then derives from the longitudinal motion.

Also the emission angles are conveniently determined by first considering the emission in the rest frame. Here usual dipole patterns are encountered and, again by a Doppler transformation, this leads in the laboratory to emission inside a narrow cone of opening angle $1/\gamma$ around the mean direction of projectile motion as in the normal bremsstrahlung case.

Figure 5b shows a collection of channelling radiation spectra recorded in the forward direction $\theta_\gamma = 0$ for 4-MeV electrons incident along the $\langle 111 \rangle$ axis in a silicon crystal. Quantum jumps are clearly visible. The positions of the peaks which vary with primary energy are fully reproducible by theoretical computations. Besides detector resolution, the finite width of the peaks is due to the finite lifetime of the electron in the various channelling states. The finite lifetime appears because the electrons, being concentrated around atomic positions, have a relatively high probability for close encounters on target atoms, displaced at random from average positions due to thermal vibration, whereby they are kicked out of their initial state of motion. Also the measured widths are in agreement with theory. It may be noted that the lengths traversed during the lifetime for all but the very short-lived ground state are $\sim 1 \mu\text{m}$, whereas photon yields per channelled electron are $\sim 1 \text{ mm}$.

The random encounters further lead to emission of radiation. Consequently, the channelling radiation spectrum is superimposed on an incoherent bremsstrahlung spectrum. The intensity of the latter is higher than for random incidence because the electrons are concentrated near target constituents. Accordingly, in Fig. 5b the $1/\hbar\omega$ -background, which survives far beyond the coherent peaks, is highest at the axis. The horseshoe like structures emerging at the higher energies are due to transitions from free states of transverse motion to bound ones. Free-to-free transitions correspond to coherent bremsstrahlung and, according to (8), they show up as straight ridges.

Patterns similar to Fig. 5b are encountered for planar channelled megaelectronvolt electrons and for positrons. For electrons, the agreement between experiments and theory is in general very good both for axial and planar cases as long as simple crystal structures are considered. For positrons, on the other hand, experimental findings have an inexplicable tendency to undershoot theoretical predictions of peak position by 5–10%.

Gigaelectronvolt impact energies

As the primary energy is raised into the gigaelectronvolt region, the number of channelling states becomes large. The level spacing reduces correspondingly. As a result, an essentially continuous transition down through the dense spectrum of states becomes possible. Hence channelling radiation appears as a classical emission process. It may be noted that by analysing this classical case, Kumakhov¹⁷ was the first to predict correctly the energy characteristics of channelling radiation through proper inclusion of relativistic effects.

Consider now, for example, a planar channelled positron. Being confined between a set of planes, this projectile oscillates transversely at a certain frequency ω_0 . The characteristics of the associated radiation may be computed in a similar way as for the coherent bremsstrahlung, (8). Alternatively, a transformation to the rest frame of the particle may be used in which case the analysis is completely parallel to the quantum case. Owing to time dilation, the oscillation frequency in the rest system attains the value $\omega_0^R = \gamma\omega_0$, and, because the motion of the positron is truly periodic here, radiation is confined to harmonics of ω_0^R . With (9), a transition to classical channelling radiation is then obtained by substituting $n\omega_0$ for $-\Delta E_\perp/\hbar$, that is,

$$\omega = 2\gamma^2 n\omega_0 / (1 + \gamma^2 \theta_\gamma^2) \quad (10)$$

where n denotes a positive integer.

In general, the value of ω_0 depends very strongly on the transverse energy of the projectile. Consequently, as a wide range of levels are populated upon entrance of the beam into the crystal, the classical channelling radiation spectra are usually

broad and featureless. However, planar positron channelling constitutes a special example. Here ω_0 depends only weakly on $E_\perp$ and, further on, in the energy region of a few gigaelectronvolts, the slight variation may even be compensated through onset of relativistic effects in the transverse motion so that all channelled positrons radiate at the same photon frequency. This requires an optimization of the positron energy towards its 'magic' value, which is specific to crystal material and orientation.

Figure 5c shows a radiation spectrum recorded for positrons moving along the $\{110\}$ planes in a silicon single crystal, at an energy close to the magic value. The intensity is normalized to the incoherent bremsstrahlung yield that is obtained for an amorphous target of the same material and thickness. A sharp peak, enhanced by a factor of 35 above the amorphous yield and corresponding to an energy of $2\gamma^2 \hbar\omega_0$, appears at approximately 30 MeV, higher harmonics being weak. The solid line shows the result of a theoretical calculation. Clearly, the agreement between experiment and theory is very satisfactory. In high-energy experiments the opening angles of photon detectors are usually large compared with $1/\gamma$. Correspondingly, the peak in Fig. 5c is sharp only on the high-energy side whereas it falls off smoothly at low energies, compare with (10). A sharp low-energy cut-off requires collimation to angles small compared with $1/\gamma$. It should be noted that the photon peak is tunable; a variation of the primary energy in the range 2–10 GeV causes the peak to move from 8 to 70 MeV. As in the quantum case, a background of incoherent bremsstrahlung survives beyond the coherent part of the spectrum. The variation of this background with incidence angle may be estimated as for the traditional close-encounter processes.

In case the transverse energy of the projectile is higher than the maximum of the planar potential, it crosses from channel to channel experiencing a kick every time the ridge (or, for the negatively charged electron, the valley) of a plane is bypassed. Denoting the angle of motion relative to the planes interspaced a distance d_p by θ the periodic kick appears at a frequency $\omega_0 = 2\pi c\theta/d_p$. Insertion of this expression into (10) leads for vanishing value of θ_γ , which here is measured relative to v , to the expression (8). The radiation emitted in the periodic crossing of continuum planes is nothing but the usual coherent bremsstrahlung.

Applications of channelling radiation

At the moment the use of channelling radiation for practical purposes is only of a speculative nature. A major part of the interest has concentrated on the prospects of constructing intense tunable X- and γ -ray sources by using the quantum peaks appearing at megaelectronvolt impact energies or the harmonic oscillator peak showing up for planar channelled gigaelectronvolt positrons, respectively. The latter case particularly provides promising results with its narrow tunable megaelectronvolt photon line and practical yields of $\sim$ one photon per incident positron. Furthermore, the obtainable degree of polarization is high.

Various applications directed towards obtaining information on crystal properties have been attempted. For instance, through the use of channelling radiation emitted by megaelectronvolt electrons, it has been demonstrated that it is possible to obtain information not only on the magnitude of thermal vibrations but, through a study of linewidths, also on the correlations between such vibrations of neighbouring atoms. It is not yet clear, however, if channelling radiation is competitive with well-established techniques for such purposes. See ref. 20 for a further discussion of possible applications.

High energies and pair production

For incidence energies in the multigigaelectron region, the classical description of channelling radiation becomes invalid. Clearly, the problem here is not a quantization of the transverse

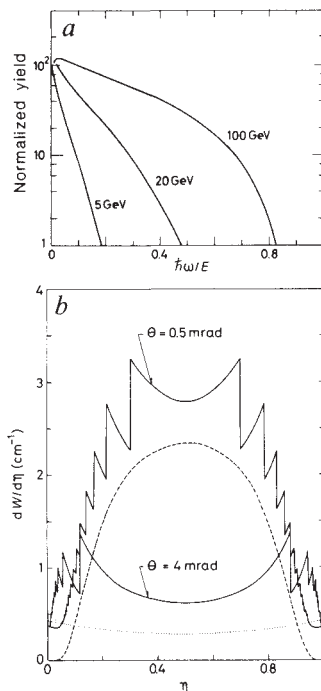


Fig. 6 Multi-gigaelectronvolt processes. *a*, Channelling radiation in the synchrotron (constant field) approximation for incidence along the $\langle 110 \rangle$ axis in silicon. Only the coherent part of the spectrum is displayed. *b*, Electron-positron pair production by 100-GeV photons incident near the $\langle 110 \rangle$ axis of a germanium crystal cooled to 100K. The quantity η denotes the fractional positron energy, $\eta \equiv E_+/\hbar\omega$.

motion of the charged particle. Instead, the breakdown is associated with the recoil of the emitted photon. The photon energy amounts to an appreciable fraction of the primary energy and cannot be neglected as in a classical treatment.

However, at these high impact energies another simplification applies: the range of photon-emission angles relative to the local direction of motion of, for example, an axially channelled electron or positron is small compared with the excursions in projectile angle relative to the axis, $1/\gamma \ll \psi_1$ ($\propto 1/\sqrt{\gamma}$). Consequently, the transverse distance travelled by the projectile during coherent photon emission is small compared with distances over which the continuum potential, or rather its derivative, varies significantly. The emission appears as in a constant electromagnetic field, a case treated in great detail in the literature and known as synchrotron radiation²⁸. To obtain the channelling radiation spectra it then suffices simply to compute those of synchrotron radiation corresponding to the various field strengths encountered in a channel and then make an average. Figure 6*a* shows theoretical spectra obtained in this way with an averaging corresponding to equal probability everywhere in the channel²². The yield has been normalized to that of incoherent bremsstrahlung. Clearly, positrons, being concentrated in the region of low field strengths, will show somewhat lower yield and photon energies than displayed in the figure whereas electrons give higher yields and energies. Channelling shows up only as a focusing or defocusing effect – exactly as in the traditional experiments, Figs 2 and 4.

Quantum mechanically, the treatment of the process where an energetic photon converts into an electron-positron pair in the field of, for example, an atomic nucleus, which is needed to ensure energy-momentum balance, is identical to that of bremsstrahlung with a few almost trivial exceptions. Consequently, the various radiation modes discussed above are paralleled by similar pair-production mechanisms.

The opening angle of the pair is $\sim 1/\gamma$. For channelling to influence the process, through capture into channelled orbits of

the charged particles produced by a photon incident along, for example, a major crystallographic axis, it is necessary that the opening angle is smaller than the critical channelling angle, $1/\gamma \leq \psi_1$. This leads to a threshold in photon energy of

$$\hbar\omega_{\text{th}} \approx 2mc^2 mc^2/|U(0)| \quad (11)$$

A typical value for, for example, silicon is 5 GeV. However, to compete with incoherent pair production it is necessary to go considerably higher in energy.

In the multi-gigaelectronvolt region it is possible to make use of the fully developed theory for pair production in strong, constant electromagnetic fields. In Fig. 6*b* the dashed curve shows the spectrum of one of the emitted charged particles obtained in this way for 100-GeV photons incident along the $\langle 110 \rangle$ axis of a germanium single crystal. The dotted curve indicates the incoherent rate and, clearly, a strong enhancement is observed. At larger angles 'coherent pair production' is approached; spectra (including the incoherent contribution) for incidence in the $\{100\}$ plane are shown as solid curves for two different angles to the axis. At the present impact energy, the spectrum corresponding to the lower angle is quite similar to the constant field result.

However, the transition with decreasing incidence angle from coherent pair production towards the region where the constant field approximation applies contains some surprises. Simple arguments on the transverse length scale involved in the production process show that the latter may be employed for angles up to an energy independent limit of approximately

$$\Theta_0 = |U(0)|/mc^2 \quad (12)$$

Above threshold, (11), this angle is large compared with the critical channelling angle, which decreases with increasing energy, $\psi_1 \propto \gamma^{-1/2}$. Hence the question whether the produced pair is channelled appears not to be decisive. Even more surprising at first glance may be the fact that applicability of the constant field approach out to Θ_0 implies a breakdown of the perturbative coherent pair production computations at angles smaller than Θ_0 (ref. 29). The breakdown appears at high energies because characteristic angles scale as $1/\hbar\omega$. This is in contrast to the result obtained at, for example, megaelectronvolt impact energies, where the coherent scheme in the case of radiation may be applied successfully all the way down to ψ_1 . However, a closer analysis shows that applicability of the perturbative approach, which produces, for example, identical radiation spectra for electrons and positrons, requires the deflection angles of the charged particles to be small compared with $1/\gamma$, the opening angle of the photon or pair-production cone²⁹. This is exactly equivalent to the condition $\Theta > \Theta_0$. The cut at Θ_0 implies a saturation of rates at ultra-high energies. Note that a typical value of Θ_0 for axial cases is $\sim \frac{1}{2}$ mrad (Ge $\langle 110 \rangle$).

In the first experiment performed to investigate the influence of the crystal lattice on multi-gigaelectronvolt pair production²² severe discrepancies from theoretical predictions of the yield were encountered. Furthermore, a dip appeared around $\sim \psi_1$. A recent second experiment³⁰ obtained much better agreement with theory and, by showing no dip at ψ_1 , it seems to support the hypothesis of applicability of the constant field approximation out to $\sim \Theta_0$. Because photons do not get focused, as opposed to the charged projectile in the radiation process, the yield appears in this approximation essentially constant out to the latter angle.

Further experiments on electron-positron pair production in single crystals are urgently awaited. When the basic phenomena are understood it may turn out that the very strong electric fields from crystal axes (or planes) may be used to catalyse rare processes – let alone the 'object' producing the electron-positron peak reported in the heavy-ion experiments at GSI, Darmstadt. As concerns applications, it is noted that the fact that the energy-independent angle Θ_0 is the decisive measure of the degree of alignment between a projectile and an axis rather than

the much smaller channelling angle ψ_1 strongly relaxes requirements to beam and crystal qualities at high energies.

Conclusions

Today it is clear that the Lindhard model, developed in the mid 1960s and based on a few basic principles, describes channelling phenomena very well over an energy range of at least eight orders of magnitude. During the past 20 years many new types of directional effects have come up ranging from low-energy electron scattering to production of rare particles in the terra-electronvolt region. The dramatic dip in yield for close encounter processes at positive charged particle impact was soon realized

as a very effective tool for many solid-state observations such as crystal disorder caused by implantation and lattice location of impurity atoms. Today, fabrication on large industrial scale by ion implantation has become of great importance.

Experimental data were recorded at the University of Aarhus and at CERN (Aarhus-CERN-Strasbourg collaboration). Similar data have been obtained elsewhere. We acknowledge the collaborations with our colleagues.

Note added in proof: A recent publication³¹ reports on a strong photon peak recorded at 85% of the primary energy for 150 GeV electrons incident parallel to the $\langle 110 \rangle$ axis in a germanium single crystal. No plausible explanation has been given for this highly interesting phenomenon.

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ARTICLES

Aerosol concentrations over the last climatic cycle (160 kyr) from an Antarctic ice core

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Time series, covering more than 150,000 years for dust and marine salt loadings in the Antarctic atmosphere have been constructed from measurements of concentrations of aluminium and sodium in an ice core taken from East Antarctica. They exhibit an inverse correlation with $\delta^{18}\text{O}$ measurements. These results extend our understanding of aerial transport processes during the last glacial cycle.

THE relation between climate and atmospheric composition over East Antarctica from the beginning of the last glacial maximum (LGM) to ~2,000 yr ago has previously been studied in detail by analysing two ice cores: the first (905 m, 30 kyr) was recovered at Dome C station (74° 39' S, 124° 10' E, 3,040 m above sea level), and the second (950 m, 50 kyr) at Vostok station (78° 30' S, 106° 54' E, 3,420 m above sea level)¹⁻⁴. A new Vostok core drilled to 2,083 m depth allowed us to extend this work over the past 160 kyr and therefore cover a complete glacial cycle. When considering continental and marine input, the last interglacial period, centred on 127 kyr BP, appears roughly similar to the Holocene. High concentration peaks observed at the end of the penultimate glacial age (around 150 kyr BP), during a cold period centred on 60 kyr ago and during the LGM may all be attributed to the same causes, i.e. great areal extent of arid source combined with stronger atmospheric circulation. For the remainder of the glacial age, from 110 to 15 kyr BP, continental and marine concentrations increase more or less regularly by factors reaching 4 and 6 respectively. For continental aerosol this may be quantitatively explained by a lower rate of snow accumulation and by sea-level lowering, leading to an

increase in the exposed area of continental shelves. For marine aerosol one should consider accumulation rate modification, greater mean wind speed over the polar ocean and more efficient transport processes over Antarctica.

Previous analyses have shown that insoluble microparticles and Al concentrations in East Antarctic ice cores are well correlated^{1,3} and that the microparticles are mainly aluminosilicates⁵. We have therefore measured Al, considering it a good continental indicator. It has been shown^{4,6} that marine aerosol, initially dominated by NaCl, loses variable quantities of Cl as gaseous HCl over East Antarctica, the remaining aerosol being mainly composed of NaCl and Na₂SO₄. Thus we considered the marine component of Na, designated Na_m (Na_m = Na - Al (Na/Al)_{crust}) (ref. 7), to be representative of the marine aerosol present as particles. Continental aerosol has different sources (crust, soils, arid soils, volcanoes), which have certainly had differing relative importances over the past 160 kyr. However, taking into account the corresponding values of the Na/Al reference ratio⁷⁻¹¹, the maximal error we made when using the crustal reference in the calculation of Na_m ranges from 5 to 17%. This is small with respect to the concentration variations we observed. Both Na

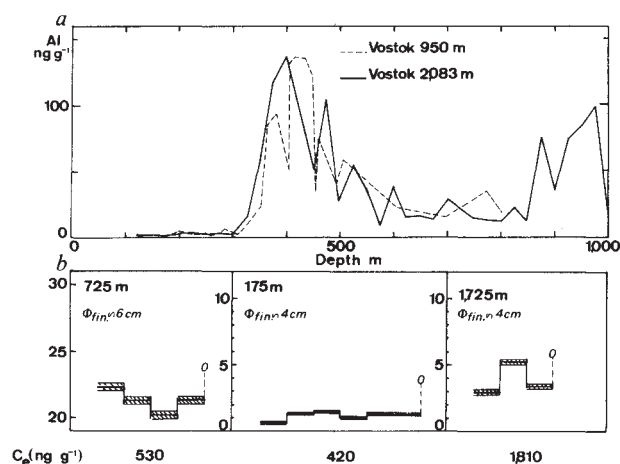


Fig. 1 *a*, Comparison of Al results obtained from the two distinct Vostok ice cores. The broken line corresponds to the 950 m core⁴ and the solid line to the first 950 m of the 2,083 m core recovered using drilling fluid. *b*, Concentrations obtained in successive melted fractions of three decontaminated samples. Those fractions correspond to ice cylinders of decreasing diameter and height and of similar thickness. ϕ_{an} is the approximate diameter of the samples after the rinsing step. 0 represents the centre of the samples and C_e the concentration measured in the outside of the original ice core. The hatched areas represent the analytical uncertainties.

and Al were measured by instrumental neutron activation with a typical analytical accuracy of 5%. Losses by adsorption or sedimentation have been checked. No loss occurs in aliquots of unacidified and vigorously shaken samples.

In contrast to the two earlier cores (Dome C, 905 m; Vostok, 950 m), which were recovered by electrothermal drilling without fluid, the 2,083-m Vostok bore hole was filled with a hydrocarbon fluid throughout drilling. This fluid was used to counterbalance the hydrostatic pressure of the ice. When drilling without fluid, meltwater is produced but quickly refreezes in the external part of the core. On the other hand, when fluid is used, hydrostatic pressure equilibrium between the interior of the core and the fluid itself, and also the thermal properties of the fluid, induce contaminant penetration farther through the ice fractures carried by the mixture of fluid and meltwater. For this reason the subsequent decontamination procedure required two steps. First, at -15°C , the ice sections were placed in an ultrasonic bath filled with acetone until the white crust formed by the refrozen mixture of fluid and water disappeared. The second decontamination step involved rinsing the samples with ultra-pure water until all fractures were removed¹. Fractures, even when annealed, remain obvious because they contain traces of fluid. When water flows on the surface of the core, escaping fluid appears iridescent, with a variety of colours depending on the number of fluid molecular beds. During fracture removal, iridescence becomes less coloured, then grey and finally vanishes.

Decontamination was checked by comparing the results obtained for the first 950 m with those published by De Angelis *et al.*⁴ and also by analysing successive melted fractions taken between the outside and the central part of decontaminated samples (principally for badly fractured samples). The results are summarized in Figs 1 and 2.

Because the two cores were recovered at the same station but at different times and by two different drilling procedures (with and without fluid), the contamination sources should have had quite different chemical compositions and physical properties. Thus the very good agreement (amplitude, relation with isotopic profiles, time dependency) between the two sets of results, one with respect to the other, as well as with Dome C data¹ cannot be a coincidence. Moreover, even in highly fractured samples (175 m), concentrations were more or less constant from the

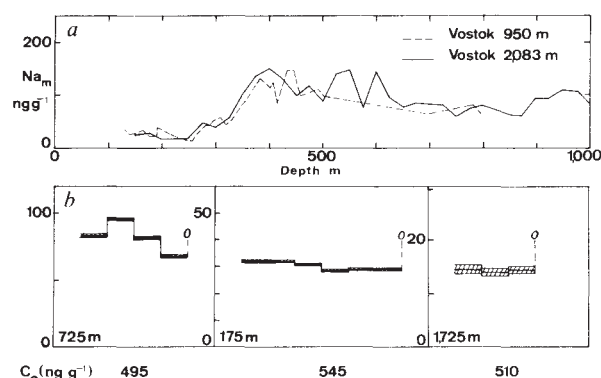


Fig. 2 *a*, As Fig. 1*a*, with Na_m instead of Al. Na_m is the marine component of Na (ref. 7). *b*, As Fig. 1*b*, with Na_m instead of Al.

outside to the centre after decontamination, the variability observed around the mean value being totally explained by the seasonal variations of both continental and marine inputs over Antarctica (reaching factors of 3 and 10 respectively^{6,12}). We therefore consider our results reliable. We analysed 91 ice sections, each covering 3–10 years (depending on their size).

We use here the chronology proposed by Lorius *et al.*¹³ and their $\delta^{18}\text{O}$ profile as a climatic reference, although no correction was made for the isotopic effect due to the variations of the ocean volume.

Results

Figure 3 displays three main features. (1) Mean concentrations of Al during the last interglacial (1,650–1,900 m depth, $3.0 \pm 1.5 \text{ ng g}^{-1}$) are not significantly different from those corresponding to the Holocene ($<250 \text{ m}$ depth, $2.5 \pm 0.9 \text{ ng g}^{-1}$). (2) Three major concentration peaks occur in phase with three of the four isotope absolute minima, the first (no. 1) corresponds to the end of the penultimate glacial age below 1,925 m depth (150 kyr BP), the second (no. 2) is centred on 900 m depth (60 kyr BP) and the third (no. 3) corresponds to the LGM at $\sim 400 \text{ m}$ depth (20 kyr BP). Peak mean concentrations are $\geq 50 \text{ ng g}^{-1}$, at least three times the background, which is the mean tendency of Al (Na_m) concentrations outside the peak areas and is represented in Figs 3 and 4 by the dot-dashed line. At the time of the fourth isotopic minimum centred on 1500 m depth (110 kyr BP), Al concentrations are not significantly different from background. (3) Over the whole of the last glacial period, the Al background gradually increases by a factor of ~ 4 .

Figure 4 shows that, except between 1,250 and 1,450 m depth, Na_m and $\delta^{18}\text{O}$ vary in opposing directions. The Na_m background gradually increases over the last glacial age by a factor reaching 6 at the end of the LGM. The four main isotope minima correspond to broad concentration peaks $\sim 50\%$ higher than background. As with Al, mean concentrations over the last interglacial period ($15.2 \pm 7.7 \text{ ng g}^{-1}$) are not significantly different from those of the Holocene ($22.8 \pm 4.3 \text{ ng g}^{-1}$).

Discussion

Studies of several East Antarctica cores⁶ have shown that, at Vostok station, dry deposition represented $\sim 60\%$ of Al and Na_m inputs during the Holocene (during the LGM the proportion of dry deposition increased to 80–90% for Al and 70% for Na_m), the remainder being removed by snowfall and nucleation processes. Thus the main features of the snow accumulation rate over the last 150 kyr¹⁴ could explain a maximum and irregular concentration increase by a factor of 2 for Al and 1.5 for Na_m during the last glacial period. Similarities in aerosol removal mechanisms under temperate and glacial climate conditions led us to think that the air-snow relationship did not vary significantly during the past 160 kyr and that the part of the

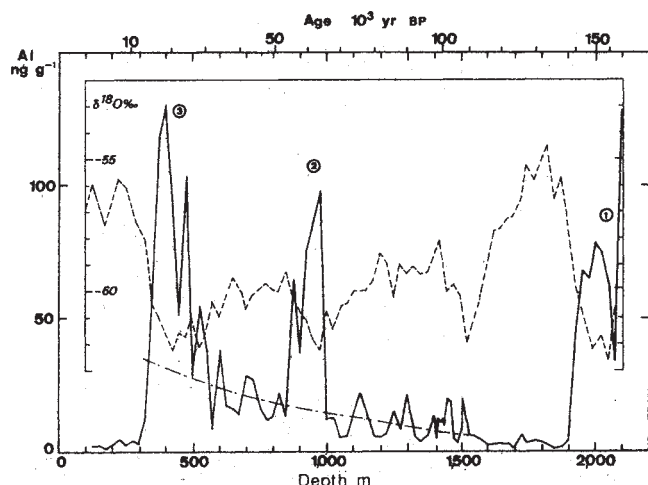


Fig. 3 Al concentration as a function of real depth (solid line). The climatic reference is given by $\delta^{18}\text{O}$ from ref. 13 (broken line). The timescale is also from ref. 13. The lower dot-dashed line represents the estimated background.

concentration increases not explained by a decrease in snow accumulation rate must be interpreted in terms of modifications of atmospheric aerosol loading.

Continental input

It has been proved^{1,2,5} that the LGM concentration peak was not of volcanic but of aeolian origin. Similarities between LGM results and events 1 and 2 (magnitude, duration and relation with isotopic data), as well as the duration of the background increase, argue in favour of an aeolian origin for the continental input over the whole period studied. Moreover the concentration of glass shards considered as an indicator of the volcanic activity did not vary significantly during events 1, 2 or 3 (A. Gaudichet, personal communication). This rules out the volcanic activity as a major cause of the variations observed.

Potential source regions for aeolian dust over East Antarctica are mainly Australia, South America¹⁵ and, during the glacial age, emerged continental shelves of the Southern Hemisphere. Indeed the sea-level lowering due to the formation of great ice caps produced 'a great broadening of South America eastwards between about 40°S and 50°S in places to almost twice its present width'¹⁶ and around Australia, southwards and northwards, the exposure of areas of nearly 30% of the present total area of the island¹⁷. Such phenomena combined with the lower snow accumulation rate could have produced an irregular increase of the continental background by a factor of 3–4 over the last glacial period, which is close to what we have observed. They cannot, however, explain the major concentration peaks.

The LGM concentration peak has been explained by both the extension of arid source areas and the intensification of atmospheric circulation^{1,3,4}. Indeed, during the LGM, owing to oceanic and land surface temperature modifications and to the emergence of large parts of the continental shelves in the Indo-Australian region which interfered with the seasonal pattern of oceanic circulation in this area¹⁸, mean winds were intensified over the Southern Hemisphere continents. Australia, Tasmania and South America (except near the Equator) were arid, semi-arid or desert^{16,18–21}. After elimination of the sea-level effect from the isotopic curve (ref. 13 and C. Lorius, personal communication) it appears that, at least over south polar regions, temperatures were similar 150, 60 and 20 kyr ago (but the fourth isotopic minimum, centred on 110 kyr BP, was not as pronounced as events 1, 2 or 3). Moreover, the ice volumes (and thus the sea level) seem to have been similar at the end of the penultimate glacial age and during the LGM. Isotopic results also suggest a rather low sea level between 70 and 60 kyr BP²². The explana-

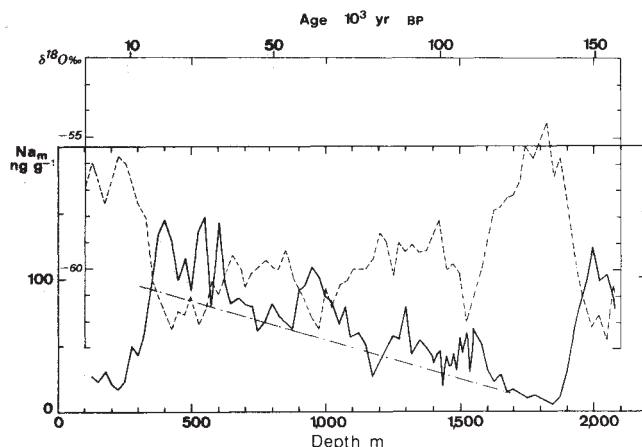


Fig. 4 As Fig. 3, with Na_m instead of Al.

tions given above for the intensification of both aridity and mean wind speed during event 3 (LGM) may thus also be valid for event 1 and partly for event 2. If so, the past 160 kyr were dominated by full glacial conditions only around 150, 60 and 20 kyr BP.

Marine input

Except for the part due to the accumulation variations, an increase in Na_m could be explained by a higher production rate, and consequently a greater wind speed, over the production areas, which are centred on 50–60°S. Such a modification of the mean wind speed could be due to both an intensified tropic-pole pressure gradient and a more southern position of the circumpolar trough, as now in early spring²³ (just as maximal marine input is observed over East Antarctica, despite its having the greatest extent of sea ice⁶). However, such meteorological modifications would have influenced the eddy diffusivity (and then the upper limit where the aerosols are transported, allowing them to travel over larger distances from the production areas) as well as the transport processes over Antarctica. Lower rates of snow accumulation during the glacial times, and also the geographical configuration of East Antarctica²⁴, appear to exclude major storm penetration over this part of the continent as a rule. Transport in the upper atmosphere was then most probably involved, as now in early spring. Although we are not able to establish the relative influence of the production rate with respect to the transport efficiency, we estimated the upper limit of the wind-speed increase^{1,25} assuming that marine aerosol input was driven only by production processes: a progressive increase of 7–10 m s^{-1} could account for the background variation, and a further increase of 1.5–3 m s^{-1} could account for the mean peak values. The proposed explanation is invalid for the broad marine event centred on 1,400 m depth (90 kyr BP), which needs further investigation, for instance by Cl/Na measurements.

This work represents the first detailed study of marine and continental inputs over Antarctica since the end of the penultimate glacial age. The modifications of the continental input seem to have been driven by meteorological conditions over tropical areas and by sea-level changes mostly linked to ice-cap formation in the Northern Hemisphere. But marine input variation over the past glacial period could be linked to subpolar, and then local, meteorological conditions. This could explain most of the differences observed between Al and Na_m concentration profiles. With regard to climate modelling, the main result suggested here is the distinction between 'full' (that is, LGM-like) and 'mean' glacial conditions in terms of production areas and wind strength. It will be tested by further analysis of continental matter composition, particle size distribution and content of major ions, for which data will soon be available.

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The $\beta\gamma$ subunits of GTP-binding proteins activate the muscarinic K^+ channel in heart

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Subunits of guanine nucleotide regulatory proteins purified from bovine cerebral cortex were used to perfuse the intracellular surface of excised patches of chick embryonic atrial cells. Single-channel current measurements unexpectedly indicate that the $\beta\gamma$, and not the α subunits, are responsible for activating the muscarinic-gated potassium channel.

GTP-BINDING proteins (G proteins) are apparently present in every cell type of higher organisms. They are a major mechanism of coupling specific receptors to cellular responses. G proteins transduce signals in systems as diverse as vision, olfaction, control of cell proliferation and ribosomal protein synthesis; they are also involved in cellular regulation by many hormones and transmitters¹. The G proteins are heterotrimers consisting of α , β and γ subunits. Recently, it has become clear that there are a number of α subunits, and it has been suggested that this diversity of α subunits is responsible for the specificity of transduction to different effectors¹. We have investigated the action of purified subunits on one such system, the muscarinic-gated potassium (K^+) current in heart. Surprisingly, $\beta\gamma$ subunits alone cause activation of single K^+ channels while α subunits do not. Thus, the α subunit may not be the only effector arm of G proteins.

Acetylcholine (ACh) is released on vagal stimulation² to bind the cardiac muscarinic ACh receptor (mAChR) and increase a K^+ current in pacemaker and atrial cells ($i_{K,ACh}$), causing heart rate to decrease³. It is not known how the mAChR is coupled to the K^+ channel but indirect evidence suggests that a G protein is involved⁴⁻⁸. Intracellular GTP is required for muscarinic cholinergic stimulation of the K^+ current⁴. When non-hydrolysable GTP analogues are used, a persistent activation of the same ACh-induced K^+ current occurs⁵. Further support comes from studies using IAP (islet-activating protein from the bacterium *Bordetella pertussis*) which ADP-ribosylates certain G proteins^{9,10}, and functionally uncouples them from their receptors. Pretreatment of cells with IAP eliminates activation of the K^+ current⁴.

Single-channel measurements of atrial cells at hyperpolarized

voltages reveal several K^+ channels which can be distinguished from each other by differences in conductance and/or gating properties. Such channels include the ATP-depletion dependent K^+ -channel, the inward-rectifying K^+ -channel and the muscarinic ACh-induced K^+ channel¹¹. The ACh-induced single-channel current ($i_{K,ACh}$) has been shown to have the same conductance but different kinetic properties when compared with the inward-rectifying single channel current (i_{K1})¹².

The inside-out mode of the patch-clamp technique¹³ is an assay system for determination of the molecular steps leading to channel activation. On formation of an inside-out patch with loss of all intracellular soluble contents (including GTP), $i_{K,ACh}$ activity markedly decreases¹⁴ even with ACh in the pipette. Addition of 100 μ M GTP to the bath (the inside surface of the patch) reactivates $i_{K,ACh}$ with ACh in the patch pipette^{6,7}. Treatment of the patch with a non-hydrolysable GTP analogue induces persistent activation of $i_{K,ACh}$ (ref. 6). Moreover, treatment of the patch with the active (A) protomer of IAP and 1 mM NAD (in a solution containing GTP) gradually eliminates $i_{K,ACh}$ (ref. 6).

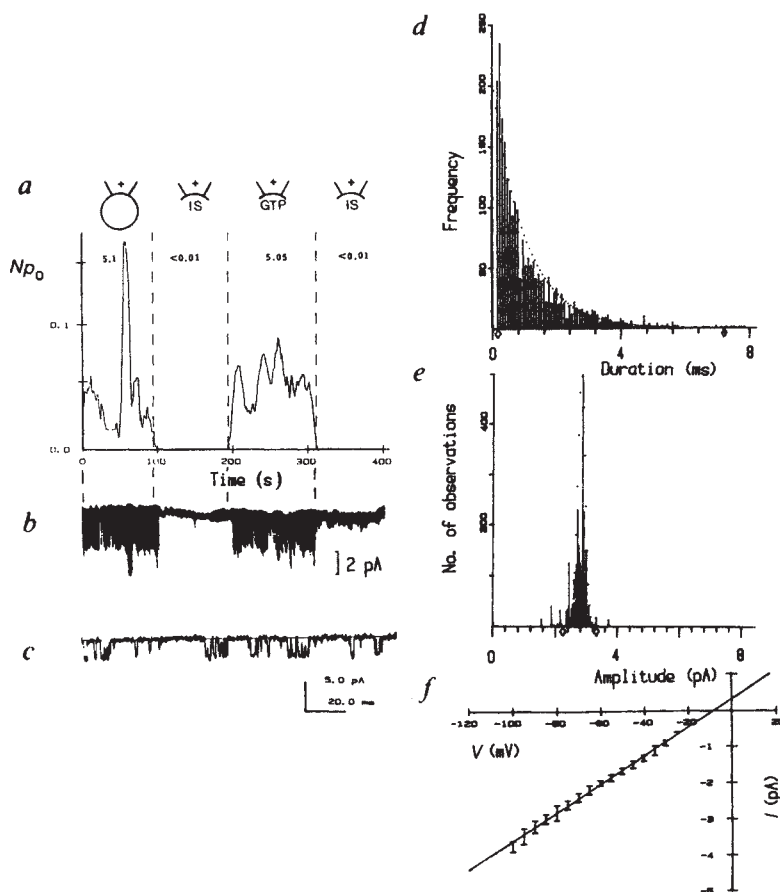
The α subunits of G proteins bind GTP and are substrates for ADP ribosylation by bacterial toxins, such as the cholera or pertussis (IAP) toxins¹⁵. There are several α subunits affected by IAP that have relative molecular mass (M_r) in the range 39,000-41,000 (α_{39} - α_{41}). IAP catalysed ADP-ribosylation of both α_{39} and α_{41} has been identified in embryonic chick heart¹⁶. The functional differences among these various α subunits are not yet known. The β and γ subunits of all G proteins are tightly associated with each other. The β subunits appear to be very similar¹⁷. However, heterogeneity does exist as β subunits occur in forms with M_r 35,000 and 36,000^{18,19} and at least two forms of γ have been described²⁰.

We have investigated the effects of the purified α_{41} , α_{39} and

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Fig. 1 Muscarinic potassium channel activation shown in cell-attached (shown schematically as circles) and inside-out (parts of circles) patch recordings from a chick embryonic atrial cell. *a, b, c*, Representative experiment ($n > 50$) showing GTP-dependent channel activity with agonist in the pipette and with or without GTP at the intracellular surface. IS, internal solution; +, ACh in pipette. *d, e, f*, Properties of the acetylcholine-induced single-channel currents. *a*, The product of the number (N) of channels in the patch and the single channel open probability (p_o) plotted as a function of time for the entire experiment (Np_o), and an overall average ($\overline{Np_o}$) is shown at the top of the plot for each condition ($\times 10^{-2}$). *b*, The primary single-channel activity is displayed on the same time scale as in *a*. *c*, Part of the initial 10 s record of *b* expanded to show representative single-channel currents. Inward current is downwards. Low-pass filtered at 2.5 kHz. *d*, Distribution of single-channel open time durations from the inside-out patch recording in GTP shown in *a* and *b*, fitted by a single exponential; decay time constant, $\tau = 1.03$ ms. For the cell-attached recording, $\tau = 1.36$ ms. *e*, Distribution of current amplitudes. Mean amplitude was 2.83 ± 0.12 pA (mean $\pm$ s.d.). Mean amplitude for the cell-attached recording was 3.00 ± 0.20 pA. Membrane potential, -80 mV (*a-e*). *f*, *i-V* relation of ACh-induced single-channel current, membrane potentials between -100 mV and -25 mV, from inside-out patches in GTP. Data from 5 different recordings were averaged for each point shown ($\pm$ s.d.). The single channel conductance was 40 pS by linear least squares fit ($r = 0.997$); reversal potential by linear extrapolation was -8 mV. The actual reversal potential was ~ 0 mV when rectification above -20 mV was considered (data not shown).

Methods. Single-channel records were measured in inside-out and cell-attached membrane patches¹³. All experiments were done at $20-22^\circ\text{C}$ on single 14-day-old embryonic chick atrial cells, enzymatically dispersed, plated at a low density, and used within 1 day in culture. The ionic composition of the solutions (denoted 'IS' for internal solution) in the pipette and bath was identical in all the experiments of this study (unless otherwise specified), and was as follows (in mM): K^+ 140; Cl^- ~ 140 ; EGTA 5; Mg^{2+} 2; HEPES 10, pH 7.2. Acetylcholine ($10 \mu\text{M}$) was present in the pipette. GTP ($200 \mu\text{M}$) was labelled as such when used. When an inside-out patch was obtained, it was brought into the mouth of a polyethylene tube of about $200 \mu\text{m}$ in diameter which, when opened, delivered test solutions to the patch at a rate of approximately $1-5 \mu\text{l s}^{-1}$. Recorded data were filtered by an 8 pole Bessel filter at 2.5 kHz and sampled at $200 \mu\text{s}$ per point by computer (INDEC). In calculating Np_o , the program first detected single-channel currents that crossed a threshold at half the expected amplitude. The area above an adjustable baseline was integrated for each opening over each time period. The total current, I , divided by the unitary current, i , gives Np_o ($Np_o = I/i$). A running average of 10 s was plotted against time for the entire experiment. Analysis of single-channel current amplitude and duration records was performed as described³⁰.



$\beta\gamma$ subunits of G proteins on activation of the K^+ channel, by perfusing the subunits onto the intracellular surface of excised patches and monitoring $i_{\text{K,ACh}}$ activity.

ACh-activated K^+ currents require GTP

ACh activation of K^+ currents in whole-cell and cell-free patches requires GTP at the intracellular surface^{4,6,7}. Figure 1*a* and *b* shows an experiment which reviews the GTP-dependence of $i_{\text{K,ACh}}$ activity for chick embryonic atria. The total current, I , passing through N channels is given by $I = Np_o i$, where i is the current through a single channel at a given membrane potential and p_o is the probability of the channel being open at that potential. Making no assumptions about the number of channels in the patch or the open probability of the channel, we used the product, Np_o , to represent $i_{\text{K,ACh}}$ activity. A running average of Np_o as a function of time (Fig. 1*a*) quantifies the real-time $i_{\text{K,ACh}}$ activity (Fig. 1*b*). Recordings of a single muscarinic K^+ channel showed typical bursting and clustering behaviour. Peaks and valleys in the plot of Np_o versus time reflected the non-stationary behaviour of muscarinic K^+ channels. Long inter-cluster intervals were quiescent periods. An overall average of Np_o ($\overline{Np_o}$) was also calculated for each experimental condition. The GTP-dependence of $i_{\text{K,ACh}}$ activity was clearly illustrated in the cell-attached and inside-out patch recordings exposed to GTP during a steady-state hyperpolarization of the membrane to -80 mV. ACh ($10 \mu\text{M}$) was present in the pipette. Channel

activity resumed in the presence of intracellular GTP and dropped to very low levels in the absence of GTP. $\overline{Np_o}$ is displayed in each sector of each figure in units of percent $\overline{Np_o}$. $\overline{Np_o}$ becomes simply the percentage open time for a single channel when $N = 1$. Figure 1*c* shows typical single-channel currents examined at higher time resolution.

Muscarinic K^+ single-channel characteristics are shown in Fig. 1*d, e* and *f*. A histogram of single-channel current open times (Fig. 1*d*) during GTP perfusion of an inside-out patch (Fig. 1*b*) was fitted best by a single exponential, which gave a decay time constant of 1.03 ms. The open time of ~ 1 ms is in close agreement with the open time reported for this channel from other cells^{6,12,14}. The amplitude of $i_{\text{K,ACh}}$, again at -80 mV membrane potential, was 2.83 ± 0.12 pA (mean $\pm$ s.d.) (Fig. 1*e*). The single-channel *i-V* relation between -100 mV and -25 mV membrane potential was linear and had a slope conductance of 40 pS, close to values reported in rabbit and guinea pig^{6,12}.

Non-hydrolysable GTP analogues

Non-hydrolysable GTP analogues induce a persistent activation of the muscarinic K^+ current^{5,6}. Figure 2*a* shows the result of the application of the non-hydrolysable GTP analogue, 5'-guanylylimidodiphosphate (Gpp(NH)p) ($1 \mu\text{M}$), to an inside-out patch with no ACh in the pipette. $\overline{Np_o}$ increased 32-fold upon application of Gpp(NH)p, and remained elevated after washout of Gpp(NH)p by internal solution, in contrast to a

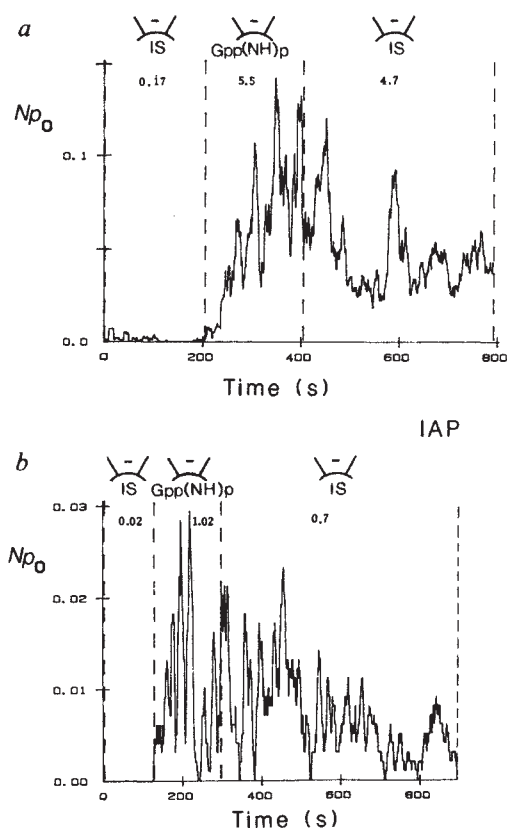


Fig. 2 Plots of Np_0 versus time for inside-out patches exposed to the non-hydrolysable analogue of GTP, Gpp(NH)p. Pipette solutions did not contain agonist (—). *a*, Application of 1 μ M Gpp(NH)p. *b*, Application of 10 μ M Gpp(NH)p to patches from an IAP-pretreated cell.

Methods. Conditions as in Fig. 1. Dissociated cells (for *b*) were treated with 250 ng ml⁻¹ of IAP just before they were plated onto culture dishes. These cells, like all others, were incubated at 37 °C and in 5% CO₂ gassed culture medium for at least 8 h before they were used.

report that the effect of Gpp(NH)p is agonist-dependent⁵. When 10 μ M Gpp(NH)p was applied to an IAP-pretreated cell (Fig. 2*b*), channel activity increased 510-fold and persisted after the perfusate was replaced by internal solution. These results are consistent with biochemical studies showing that adenylyl cyclase can still be inhibited by Gpp(NH)p-liganded G protein even after IAP-induced ADP ribosylation²¹. Thus, non-hydrolysable GTP analogues can activate $i_{K,ACh}$ in the absence of agonist⁶ and after pertussis toxin-mediated ribosylation of the α subunit.

Role of G protein subunits

Inside-out patches were perfused with purified α_{39} , α_{41} and $\beta\gamma$ subunits from bovine cerebral cortex¹⁸ to test which subunits affect $i_{K,ACh}$ activity. The internal solution also contained 40 μ M dithiothreitol (DTT) and 184 μ M 3-[(3-cholamidopropyl)-dimethylammonio] 1-propane sulphonate (CHAPS) to prevent protein degradation and aggregation (Fig. 3, legend). The detergent concentration used did not disrupt the membranes and inside-out patch recordings were stable for long periods of time (0–2.5 h). Control experiments with these concentrations of CHAPS and DTT in internal solution alone had no effect on patch current. Figure 3*a* shows a one-dimensional SDS-polyacrylamide gel of the purified subunits used.

All our experiments designed to activate the $i_{K,ACh}$ channel with free α_{39} and α_{41} were negative. In these studies, we used α -GDP as well as activated α (α -GTP γ S or α -Gpp(NH)p). The fact that GTP γ S and Gpp(NH)p stimulated endogenous

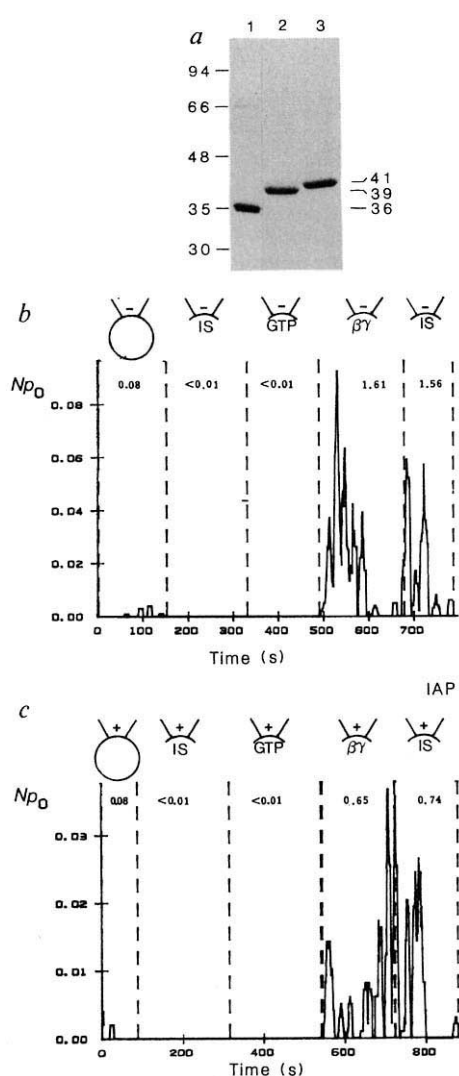
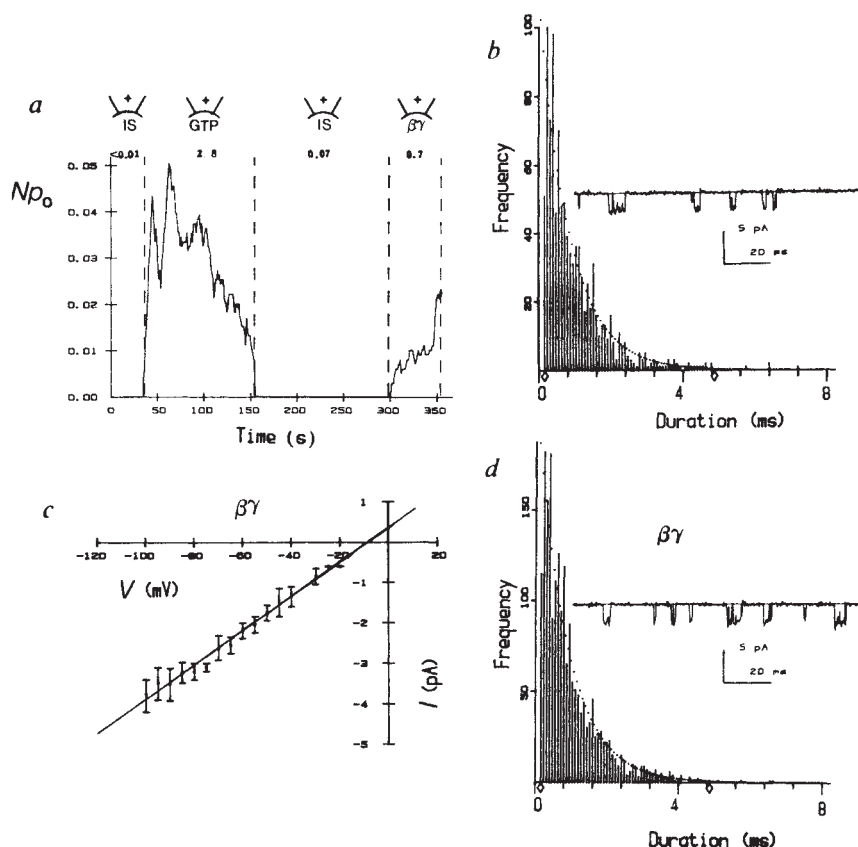


Fig. 3 *a*, One-dimensional SDS-polyacrylamide gel stained with Coomassie Blue showing purified $\beta\gamma$, α_{39} and α_{41} components and *b*, *c*, time plots of channel activity (Np_0) from cell-attached patches and inside-out patches exposed to the purified $\beta\gamma$ subunits of *a*. *a*, The β subunit (lane 1) was a doublet of proteins of M_r 35,000 and 36,000. The γ subunit is found in the dye front; α_{39} and α_{41} are in lanes 2 and 3, respectively. Mass of each protein loaded was 2 μ g. *b*, Effect of $\beta\gamma$ complex applied at 1.0×10^{-3} g l⁻¹ (23 nM; $\beta\gamma$ M_r 44,000). *c*, Application of $\beta\gamma$ complex (23 nM) to an inside-out patch from an IAP-pretreated cell.

Methods. Purification was as described¹⁸, except that the purified proteins were re-run through a DEAE-Sephacel column in 0.3% CHAPS. This modification was necessary because both Lubrol and cholate cause patch membrane breakdown. The purified protein was dialysed for ≥ 6 h with internal solution containing 0.3% (or 4.6 mM) CHAPS and 1 mM DTT. The final concentrations of CHAPS and DTT used in all experiments with purified proteins were kept constant at 0.012% (or 184 μ M) and 40 μ M respectively. A sample of the dialysed pure protein was subjected to amino-acid analysis to determine final concentration³¹.

activity made it difficult to determine the effect of GTP γ S or Gpp(NH)p liganded α subunits. To circumvent the problem, we preincubated α_{41} and α_{39} with 200 nM GTP γ S for 30 min at 22 °C and diluted the protein 20-fold immediately prior to use. The final concentration of GTP γ S (or Gpp(NH)p) was 10 nM and the concentration of α was either 25 nM (α_{41}) or 64 nM (α_{39}). In controls, this low concentration of the non-hydrolysable GTP analogues alone did not activate the channel. Thus, neither α -GDP nor α -GTP γ S activated the channel. It

Fig. 4 Properties of $\beta\gamma$ -induced single-channel currents (*a, c, d*) and currents induced by the native G binding proteins with no IAP pretreatment (*a, b*). Conditions as in Fig. 1. *a*, Channel activity from an inside-out patch where either the endogenous or exogenous subunits of the G proteins activated $i_{K,ACh}$, thus affording direct comparison of their effects on Np_o ; $[\beta\gamma] = 55$ nM. *b*, Distribution of single channel open time durations from the inside-out patch recording exposed to GTP as in *a*, fitted by a single exponential with decay time constant 0.88 ms. This compares well with the distribution of Fig. 1*d* which gave a decay constant of 1.0 ms under identical conditions. Sample single-channel currents are shown in the inset. *c*, i - V relation of the $\beta\gamma$ -induced single-channel current between -100 mV and -20 mV membrane potential from inside-out patches. Data from 5 different recordings were averaged ($\pm$ s.d.). The single-channel conductance was 42 pS by linear least squares fit ($r = 0.99$). Extrapolated reversal potential, -7 mV. *d*, Distribution of single channel open time durations from the inside-out patch recording exposed to $\beta\gamma$ as shown in *a*. The distribution is fitted by a single exponential; decay time constant, 0.86 ms. Sample single-channel currents are shown in the inset.



is, of course, possible that α alone may not insert properly into the lipid bilayer²² and our results do not completely rule out a direct role for α subunits in K^+ channel gating.

In contrast, perfusion of a patch with purified $\beta\gamma$ subunits (23 nM) increased the frequency of channel openings by at least 150-fold without ACh in the pipette (Fig. 3*b*). Even after removal of $\beta\gamma$ from the perfusate, activity remained elevated. Figure 3*c* shows that IAP pretreatment of a cell functionally uncoupled the muscarinic receptor from the K^+ channel, because application of GTP at the intracellular surface did not activate $i_{K,ACh}$ even with ACh in the pipette. Again, application of purified $\beta\gamma$ subunits (23 nM) resulted in a persistent channel activation. We used $\beta\gamma$ subunits from two different purification procedures and obtained consistently similar results.

The experiment shown in Fig. 4*a* compared the activity of K^+ channels regulated by the native G proteins with that induced by the purified $\beta\gamma$ complex (same patch). In the presence of ACh, perfusion of GTP activated $i_{K,ACh}$ for as long as GTP was available. Perfusion of purified $\beta\gamma$ subunits also stimulated $i_{K,ACh}$. A histogram of single channel current open times at -80 mV membrane potential in 200 μ M GTP (Fig. 4*b*) was fitted by a single exponential with a decay time constant of 0.88 ms. The open time histogram derived from channels in $\beta\gamma$ alone was fitted by a single exponential with a decay time constant of 0.86 ms (Fig. 4*d*).

For $\beta\gamma$ -activated channels, the single channel i - V relation between -100 mV and -20 mV membrane potential was linear with a slope conductance of 42 pS (Fig. 4*c*, 5 cells). Since both the gating and conductance properties of the channel activated by the $\beta\gamma$ subunits are identical to the muscarinic K^+ channel, we conclude that the $\beta\gamma$ subunits activated $i_{K,ACh}$.

Specificity of $\beta\gamma$ effects

We performed several experiments to demonstrate the specificity of K^+ channel activation by $\beta\gamma$. To rule out the possibility that the $\beta\gamma$ -mediated stimulation was due to a non-protein impurity, boiled $\beta\gamma$ was used in control experiments. Perfusion of boiled

$\beta\gamma$ did not activate channels in a patch in which unboiled $\beta\gamma$ did (data not shown, $n = 3$).

The subunits of the $\alpha\beta\gamma$ heterotrimer are in equilibrium as follows:



If the active form is free $\beta\gamma$ and the $\alpha\beta\gamma$ trimer is incapable of activating $i_{K,ACh}$, then at high $[\alpha\text{-GDP}]$ to $[\beta\gamma]$ ratios, there should be no channel activity. We tested this hypothesis with various ratios of $[\alpha\text{-GDP}]$ to $[\beta\gamma]$ in the solution for both α_{39} and α_{41} . The results of 15 experiments showed that when $\beta\gamma$ is preincubated with a sufficient excess of α , $i_{K,ACh}$ is not stimulated. In each of these experiments, control perfusion of $\beta\gamma$ alone triggered $i_{K,ACh}$ activity. Figure 5*a* and *b* shows two experiments in which an excess of α_{41} (98 nM) was first preincubated with $\beta\gamma$ (23 nM) and the combination used to perfuse inside-out patches. This $\alpha_{41}\beta\gamma$ solution failed to stimulate $i_{K,ACh}$. However, $i_{K,ACh}$ was clearly activated when the same patch was perfused with an identical $\alpha_{41}\beta\gamma$ solution, in which the α_{41} had first been boiled before it was allowed to react with $\beta\gamma$. The K^+ channel activation persisted even when the purified proteins were washed out by internal solution. As before, the effect did not require ACh (Fig. 5*a*) and IAP pretreatment did not prevent activation (Fig. 5*b*). We therefore conclude that $i_{K,ACh}$ stimulation is specifically due to $\beta\gamma$ and that the $\alpha\beta\gamma$ trimer fails to activate the K^+ channel. If α_{41} has a higher binding affinity for $\beta\gamma$ than does α_{39} (ref. 23), relatively less α_{41} than α_{39} should be needed to react with a fixed amount of $\beta\gamma$ to shift the equilibrium of reaction (1) to the right. In a series of experiments, we compared the effects of various ratios of α to $\beta\gamma$ on channel activation, keeping $[\beta\gamma]$ constant. For $i_{K,ACh}$ activation, less than half the amount of α_{41} compared with α_{39} was needed to prevent channel activation by $\beta\gamma$ (both in the same patch and in different patches; data not shown, $n = 11$).

Gpp(NH)p has been shown to activate G proteins by causing dissociation of the purified $\alpha\beta\gamma$ trimer in solution²⁴⁻²⁶. Since Gpp(NH)p can activate the endogenous G proteins in the patch

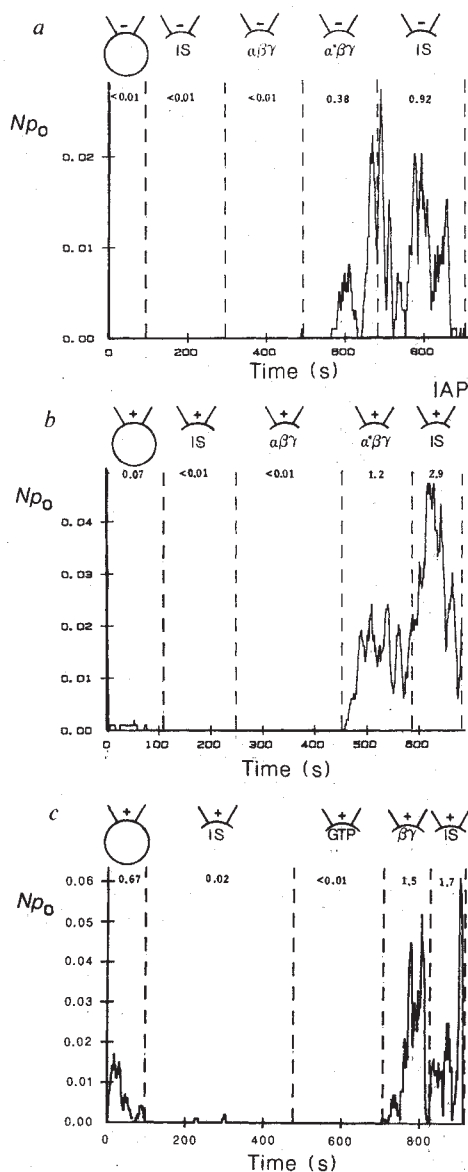


Fig. 5 Activity of K^+ channels from cell-attached and inside-out patches exposed first to non-boiled α_{41} (98 nM) preincubated with $\beta\gamma$ (23 nM) and then (a, b) to boiled α_{41} (denoted as α^*) preincubated with $\beta\gamma$ and (c) to Mg^{2+} -free solutions. a, Application of $\alpha_{41}\beta\gamma$ (ratio $\alpha:\beta\gamma=4.3:1$) followed by $\alpha^*\beta\gamma$. K^+ channel activation took place in the absence of ACh in the pipette (as in Fig. 3b). b, Same protocol as (a): $\alpha^*\beta\gamma$ activated $i_{K,ACh}$ in a cell pretreated with IAP (as in Fig. 3c). c, $\beta\gamma$ Activates $i_{K,ACh}$ in Mg^{2+} -free solution; $[\beta\gamma]=55$ nM.

Methods. Purified α_{41} and $\beta\gamma$ (or α_{39} and $\beta\gamma$) were preincubated at 22 °C for 15 min at μM concentrations. They were then diluted to nM concentrations keeping their relative ratios constant to levels where $\beta\gamma$ worked routinely when applied alone (23 nM). The α^* had been boiled for 5 min before preincubation with $\beta\gamma$. In c, dialysis of the purified $\beta\gamma$ complex was performed with Mg^{2+} -free internal solution containing 1 mM DTT and 0.3% (or 4.6 mM) CHAPS.

and thus stimulate $i_{K,ACh}$, it was not possible to decide whether Gpp(NH)p also activated the purified $\alpha\beta\gamma$ trimer. GTP does not cause $\alpha_{39}\beta\gamma$ or $\alpha_{41}\beta\gamma$ dissociation in solution²³ and similarly we could not activate $i_{K,ACh}$ by adding GTP to purified $\alpha_{39}\beta\gamma$ or $\alpha_{41}\beta\gamma$.

The effect of $\beta\gamma$ does not require Mg^{2+}

It has recently been shown that activation of $i_{K,ACh}$ by GTP or non-hydrolysable GTP analogues has an absolute requirement

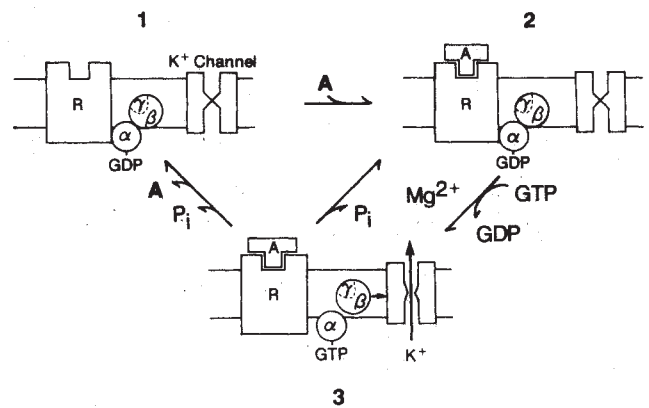


Fig. 6 Simplified scheme of activation of the $i_{K,ACh}$ channel. R, membrane receptor (muscarinic ACh); A, ACh; α , β , γ are the three subunits composing G proteins. See text for explanation. State 2 is introduced to simplify conceptually the events of agonist binding and GTP binding, without implying that this is either the only state between 1 and 3 or that there is a measurable time interval that separates agonist binding from GTP binding.

for Mg^{2+} (ref. 8). Dissociation of the trimer into α and $\beta\gamma$ subunits is also Mg^{2+} -dependent^{27,28}.

In the experiment shown in Fig. 5c, all solutions used were Mg^{2+} -free. During the cell-attached recording, when Mg^{2+} and GTP were present on the intracellular side of the membrane, $\bar{Np}_0$ was 0.67. After inside-out patch formation, all water soluble molecules were perfused out of the bath (including GTP and Mg^{2+}). Reperfusion with an Mg^{2+} -free, GTP-containing solution did not activate $i_{K,ACh}$ despite the presence of ACh in the pipette. However, purified $\beta\gamma$ perfusion in Mg^{2+} -free solution caused a 150-fold persistent increase in $\bar{Np}_0$. This Mg^{2+} -free activation of $i_{K,ACh}$ suggests that Mg^{2+} is required for a step prior to the interaction of $\beta\gamma$ with the K^+ channel. In the absence of Mg^{2+} , the muscarinic K^+ channel no longer rectifies and clear reversal potentials can be measured. This suggests a role for Mg^{2+} in permeation of this channel as well.

Discussion

That Gpp(NH)p can activate $i_{K,ACh}$ suggests that G proteins couple muscarinic receptors to the K^+ channel. The activation by non-hydrolysable analogues occurs distal to the receptor since it occurs in the absence of agonist or when the receptor is uncoupled from G proteins by pertussis toxin-catalysed ADP-ribosylation²⁹.

As G proteins are made up of heterologous subunits, we sought to determine the role of individual subunits in regulation of K^+ channel activity. To do so, we used purified α_{39} , α_{41} and $\beta\gamma$ subunits to perfuse inside-out patches in which the muscarinic receptors and K^+ channels had been uncoupled by (1) not including ACh in the pipette; or (2) ADP-ribosylation of endogenous α proteins. To our surprise, the purified $\beta\gamma$ complex alone was able reproducibly to increase $i_{K,ACh}$. Neither α -GDP nor α -GTPyS (α_{39} or α_{41}) were able to activate $i_{K,ACh}$. When the α subunits were added in excess over $\beta\gamma$ they inhibited the activation of the K^+ channel by $\beta\gamma$. Heat-inactivated α that had been preincubated with $\beta\gamma$ did not inhibit the activation by $\beta\gamma$, implying a specific effect by α on $\beta\gamma$. The inability of the $\alpha\beta\gamma$ trimer to activate the channel suggests that the activation of the G protein depends on dissociation of the $\alpha\beta\gamma$ trimer into its α and $\beta\gamma$ subunits. This conclusion is supported by the observation that Mg^{2+} is necessary for activation and dissociation of the G proteins but it is not necessary for $i_{K,ACh}$ activation by free $\beta\gamma$.

Figure 6 shows a model for muscarinic K^+ channel activation that incorporates all our findings. When ACh binds its muscarinic receptor, GTP can interact with the α subunit of a G protein (α_{41} or α_{39}). Then, in an Mg^{2+} -dependent manner, the $\alpha\beta\gamma$ trimer becomes activated (possibly undergoing subunit

dissociation into its α and $\beta\gamma$ subunits) allowing $\beta\gamma$ to stimulate $i_{K,ACH}$. Presumably, the hydrolysis of GTP (allowing reassociation of the subunits) inactivates the G protein and allows the channel to return to basal levels of activity. Since adenosine activation of the muscarinic K^+ channel has been shown to be GTP-dependent⁶, it is possible that the same mechanism underlies the action of adenosine.

Our model raises a number of questions. For example, do the $\beta\gamma$ subunits interact directly with the K^+ channel or are there other intermediates? An indirect action of $\beta\gamma$ on $i_{K,ACH}$ would have to occur through a membrane-bound intermediate to account for long-term activity in vigorously perfused cell-free patches. Also, such an intermediate could not use ATP as a substrate for phosphorylation since we have excluded ATP from the perfusate with no change in the effect. One possible model, in which $\beta\gamma$ overwhelms a tonic inhibitory effect of α on $i_{K,ACH}$, cannot be rigorously excluded although it is not the simplest explanation of the data. In the unactivated state, the G proteins probably exist as associated heterotrimers. Therefore, the putative inhibition of the K^+ channel in the basal state would be due to association of a heterotrimer with the channel. To relieve a tonic inhibitory effect of α on $i_{K,ACH}$, $\beta\gamma$ would have to displace not free α but an $\alpha\beta\gamma$ complex. These and similar considerations make us favour a model in which $\beta\gamma$ activates $i_{K,ACH}$ directly.

Although $\beta\gamma$ seems to be the active agent in these studies, activation of a G protein by the muscarinic receptor may liberate α subunits as well. It is possible that α subunits are also involved in regulating the channel even though we could not demonstrate direct activation. For instance, α might inactivate (or desensitize) the K^+ channel after $\beta\gamma$ activation. The activated α protein

might also interact with other membrane enzymes or proteins: it might antagonize the activation of adenylyl cyclase by the β -adrenergic receptor, inhibiting calcium channel phosphorylation and decreasing net calcium current⁵. Thus, activation of the $\alpha\beta\gamma$ trimer might liberate two effectors, with the ultimate result being a change of action potential duration and a slowing of the heart rate.

The model depicted in Fig. 6 leaves unsettled the issue of agonist specificity. How does a specific agonist like ACh activate only $i_{K,ACH}$ since free $\beta\gamma$ can be released by various G proteins? One possibility is that $\beta\gamma$ subunits are not all equivalent. Indeed, evidence exists for a variety of $\beta\gamma$ subunits of similar, but not identical, M_r ^{18,19}. Subtle differences in the structure of these subunits would enable them to interact only with specific molecules. In this case, our finding that bovine brain $\beta\gamma$ could activate a heart K^+ channel could be merely a reflection of the heterogeneity of our purified $\beta\gamma$ protein. A second possibility is that the receptor, G protein, and K^+ channel are physically close so that small conformational changes in one component are rapidly communicated to the others. Finally, perhaps the differing binding affinities of α subunits to the $\beta\gamma$ subunits may play a role in creating agonist specificity. Definitive answers to these questions may come from reconstitution of purified components of this system in artificial lipid bilayers.

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LETTERS TO NATURE

Nucleus of comet Halley as a torque-free rigid rotator

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Recent observations provide evidence for approximately 2.2 day and 7.4 day periods in phenomena associated with the nucleus of comet Halley. Here I propose a model for the rotation of the nucleus on the assumption that it is homogeneous and a torque-free rigid rotator. It spins about its long axis with a period of 7.4 days, while this axis precesses about a fixed direction with a period of 2.2 days. To satisfy the moments of inertia, the precession angle is to be 77°. This model settles the major problems associated with the recent controversy of two rotation periods.

The evidence for a periodicity of 52 to 53 hr or about 2.2 days was based on observations made in both 1910 and 1985-86 and included the recurrence of dust jets and outbursts detected on the comet's Earth-based images and the repeating appearance of the hydrogen halo detected by the Lyman- α imager on the Suisei mission when on the way to Halley. A list of these recurring events was compiled by Sekanina¹. The 2.2-day periodicity was subsequently confirmed by close-up imaging of the comet's nucleus from the Vega missions².

More recently, Millis and Schleicher³ have presented the results of their spectrophotometric observations of Halley on 37 nights in March and April 1986, finding strongly pronounced double-peak brightness variations with a period of 7.4 days. M. C. Festou (personal communication) confirms this periodicity in various sets of observations, including those from the International Ultraviolet Explorer (IUE).

During the 20th ESLAB Symposium on the Exploration of Halley's Comet, Heidelberg, West Germany, late October 1986,

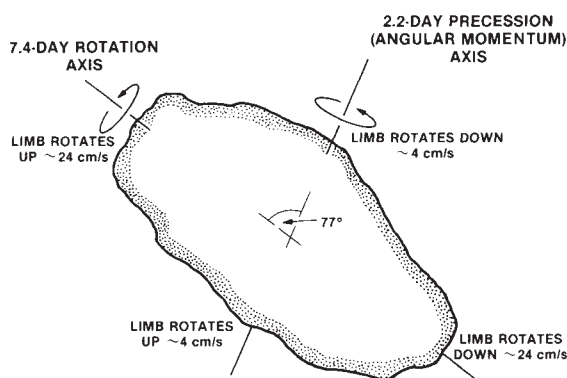


Fig. 1 Schematic representation of the modelled rotation of comet Halley.

and later during the 18th Annual Meeting of the Division for Planetary Sciences of the American Astronomical Society, Paris, France, controversy concerning the two rotation periods arose. The general feeling was that we have to consider both the rotation and wobbling of the comet's nucleus, but opinions that one of the two periods is simply incorrect were also voiced.

A dynamically significant property of Halley's nucleus is its basic outlines, with one long and two short body axes in an approximately orthogonal configuration, with ratios of 1.9:1:1 (ref. 2). Unlike an oblate spheroid, the most probable position of the rotation axis is not immediately obvious. The situation is further complicated by the lack of any information on the structure and mass distribution in the comet's interior, so that the moments of inertia about the three axes can only crudely be estimated. In addition, the highly nonspherical shape of the nucleus and the strongly collimated jets as seen on the Giotto images⁴ suggest that the torque exerted on the nucleus by outgassing may not be negligible. We are confronted with an extremely complex scenario, which should be essentially understood before we proceed with refined mapping of dust sources on the nucleus surface^{5,6} and before we can interpret fully the *in situ* dust-impact curves recorded by the various spacecraft (for example, refs 7, 8) and the Doppler-shift measurements of the time profile of the momentum transferred by dust particles to the Giotto spacecraft⁹.

A first step in this long process is made below. The major objective of this paper is to accommodate the 2.2-day and 7.4-day periods into a self-consistent formalism, by presenting a simple, first-approximation model, in which Halley's nucleus is assumed to be homogeneous and a torque-free rigid ellipsoid, which rotates about its centre of gravity and whose moments of inertia about the two short axes, x and y , are identical. On these assumptions Euler's equations can be written as

$$\begin{aligned} I_0 \dot{\omega}_x &= (I_0 - I_z) \omega_y \omega_z \\ I_0 \dot{\omega}_y &= -(I_0 - I_z) \omega_x \omega_z \\ I_z \dot{\omega}_z &= 0, \end{aligned} \quad (1)$$

where ω_x , ω_y , and ω_z are the angular velocities about the three orthogonal axes in the body coordinates, I_0 is the moment of inertia about the axes x and y , and I_z is that about the long axis z . The solution, in terms of the Euler angles, is

$$\begin{aligned} \omega_x &= \dot{\psi} \sin \theta \sin \psi t \\ \omega_y &= \dot{\psi} \sin \theta \cos \psi t \\ \omega_z &= \dot{\psi} + \dot{\phi} \cos \theta = \dot{\phi} (I_0 / I_z) \cos \theta \end{aligned} \quad (2)$$

where t is time, $\dot{\psi}$ is the spin rate about the axis z , $\dot{\phi}$ is the angular rate at which this axis is precessing about a fixed axis

in space, and θ is the angle between the two axes or the precession angle. The constants $\dot{\psi}$, $\dot{\phi}$, and θ are interrelated by

$$\dot{\psi} = \dot{\phi} (I_0 / I_z - 1) \cos \theta \quad (3)$$

The moments of inertia are

$$\begin{aligned} I_0 &= (a^2 + b^2) M / 5 \\ I_z &= 2b^2 M / 5 \end{aligned} \quad (4)$$

where M is the mass of the nucleus and a and b are its semimajor and semiminor axes, respectively. Equations (3) and (4) combine to give

$$\cos \theta = (2\dot{\psi} / \dot{\phi}) / [(a/b)^2 - 1] \quad (5)$$

From ref. 2 one finds that $a/b \approx 1.9$. If the rotation period about the long axis is identified as 2.2 days and the precession period as 7.4 days, relation (5) gives no solution. On the other hand, if the two periods are interchanged, then $\dot{\psi} / \dot{\phi} \approx 0.30$ and $\theta \approx 77^\circ$. It is noticed that the precession of the long axis can be approximated rather closely by a rotation about an axis in the 'waist' of Halley's nucleus, which would correspond to $\theta = 90^\circ$. The kinetic energy of rotation per unit mass of the nucleus is 74 erg g^{-1} .

The simple assumption of a torque-free rotator for Halley's nucleus leads to a self-consistent model in which the 2.2-day period is assigned to the precession of the long axis of the nucleus and the 7.4-day period to the rotation about this axis. The moments of inertia require that the precession angle of the long axis be 77° , which explains why Halley's wobbling was in many previous studies rather successfully approximated by assuming 'pure' rotation with the period of 2.2 days.

The presented solution (Fig. 1) is also attractive for several other reasons. One point now fully understood is the incompatibility¹⁰ of the shape and orientation of the nucleus (as derived from its combined close-up imaging during the Vega 1, Vega 2, and Giotto encounters) with the 7.4-day period. This is the case because the aspect changes are determined by the precession and are therefore synchronized with the 2.2-day period.

An interesting problem is that of variations in the comet's brightness. Photometry is, unfortunately, less diagnostic (especially when made with relatively large apertures) than direct imaging because it does not allow the determination of the one-to-one correspondence among recurring features or events. Because of the 7.4-day light curve's double-peaked shape, the possibility was investigated whether this period could in fact be a composite of an intrinsic rotation period about the long axis and the 2.2-day period. No satisfactory solution has been found, but this problem should be reinvestigated when all the relevant observations become available. One would expect a greater effect from the overall nucleus shape at large heliocentric distances, where the 2.2-day period ought to prevail. Near the Sun the interplay between the two periods reflects the surface distribution of the activity sources. Discrete areas near the ends of the long axis obviously emphasize the 2.2-day periodicity. On the other hand, significant elevation variations over the nucleus surface (that is, local deviations from the assumed ideal figure) can cause emission sources at certain locations to be subjected to rather minor insolation variations during the 2.2-day precessional cycle, while their activity experiences major changes during the rotation about the long axis. The two-hump light curve of Millis and Schleicher³ may reflect these local regolith irregularities. Variations with the orbital position in the angular distance of the Sun from the precession axis and in the precession angle (nutation) should also be of consequence. Finally, the fact that Millis and Schleicher's observations show the 7.4-day periodicity to be much more pronounced in the gas emission (such as C_2 and CN) than in the continuum may signal differences in the surface distributions of outgassing and dust production.

Interpretation of imaging sequences of jets should primarily be concerned with the 2.2-day precession period, which—being the shorter of the two—usually determines the initiation and termination of jets. However, the rotation about the long axis obviously modifies the distribution of available dust-emission sources during consecutive precession periods, which is essential for the full understanding of the pattern of jet activity and for mapping the sources on the nucleus surface. The need is implied for reinterpretation of the results based on photographs from 1910⁸. The rotation about the long axis can now account for numerous instances of 'missing jets,' when a previously established dust source was found to become 'dormant' during the subsequent cycles. The concept of an insulating mantle, formerly employed to explain the state of 'dormancy,' is now clearly deemphasized. Effects of the 7.4-day modulation are likely to explain other properties of the pattern of the source distribution as well, including short-duration activation of some sources (such as those responsible for the 'antisunward' jets) and the occasional appearance of jets from 'high-latitude' regions.

The 77° precession angle of the long axis implies a total wobble amplitude of $(90^\circ - 77^\circ) \times 2 = 26^\circ$ for what previous studies interpreted as the 'spin axis.' Hence, the fact that its various determinations appear to have placed the pole within a few tens of degrees of one another is no longer found to be disturbing.

In conclusion, one should address the question of why Halley could rotate (at least in the first approximation) in this relatively unstable configuration (prolate spheroid). One can only speculate that this may be a result of the comet's nucleus splitting or of a major impact event, perhaps a collision with a relatively massive object, in the not too distant past. Also, there may be a contribution from the torque effects by outgassing, which

would of course violate the assumption of free precession. In any case, it appears that the rate of damping of Halley's wobbling motion by internal energy dissipation is insignificant on a time scale comparable to that of the suggested events and that, therefore, this comet's nucleus appears to be rather a rigid body. Any future refinements of this crude, first-approximation model may of course require changes in some of the present conclusions.

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Note added in proof: Numerical experimentation (still in progress) shows that a light curve with a single, double, or triple hump during the 7.4-day period can be explained by emission from a solitary discrete source, if the precession and rotation periods are in or near a 3:1 resonance. The number of humps and their separations depend on the source's location on the nucleus surface.

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Novel materials from protein-polymer grafts

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Proteins are the most underrated and under-used polymers: their impressive properties include infusibility, great mechanical strength and inherent adhesive capability due to a highly flexible backbone and many functional side chains. The notion of moisture sensitivity of proteins is not universally true. Barnacle cement (which can adhere to Teflon) and mussel and clam byssus, all of which are 99% protein, set in the presence of water and resist enzymatic as well as chemical degradation at ambient temperature. This observation suggests that proteins that are capable of tight three-dimensional cross-linking can overcome sensitivity to moisture and enzymatic attack. It should then be possible to achieve similar resistance by appropriate chemical manipulation of proteins, leading to cross-linking. We have achieved such a result with an ordinary protein, commercially available gelatin, which was chemically modified and then epoxidized. When cured such a material binds to metals and plastics. Any protein that has modifiable amino acids can be used for this purpose.

There have been numerous studies directed to grafting of various monomers onto different insoluble, fibrous materials such as silk^{1,2}, cotton³, wool^{4,5} with the intention of improving dyeability, moisture resistance, and so on. In no case was there evidence that these modifications were carried out to produce materials of construction. The gelatin-epoxide graft described

here is the first example of a novel class of materials that do not exist in nature. Furthermore, they represent materials formed from a renewable resource. Another member of this class, a gelatin-phenolic graft, will be described elsewhere. The uniqueness of these materials stems from the fact that the protein is selectively modified to increase the number of sites for grafting. Furthermore, the proportion of the protein to polymer can be varied at will: the graft described here has ~5 wt% polymer; however, by repeating the epoxidation this ratio can be increased to 50 or 75%. One can also graft pre-formed polymers or produce grafts with more than one polymer, stepwise or simultaneously. In each case, the function of the polymer is to provide three-dimensional cross-linking.

The protein chosen for our study was commercially available 300 Bloom gelatin. Its backbone was modified by reductive arylation, using phenolic aldehydes. On this modified backbone an epoxide resin was grafted stepwise. The graft was cured using classical epoxide-resin curing techniques⁶. The procedure followed is outlined in Fig. 1.

In reductive alkylation⁷⁻⁹, an amine is reacted with an aldehyde to form a Schiff's base. Because of the presence of a double-bond, Schiff's bases are not very stable but this problem is solved by the use of an appropriate reducing agent, yielding a substituted methylene amine. Because our aim is to form an epoxide resin on the protein backbone, we chose phenolic aldehydes, 4-hydroxy, 2,3- 2,4- and 3,4 dihydroxy benzaldehydes, to modify the lysines with sodium cyanoborohydride as the reducing agent. To indicate the exclusive use of aromatic aldehydes in this procedure it was termed 'reductive arylation'. Several steps, corresponding to the following reactions were used to obtain the grafts.

For the modification of gelatin backbone 5 g gelatin corresponding to 200 mg of lysine (1.408 mM) was dissolved in 70 cm³ borax buffer (pH 7.2). Ten molar excess of phenolic aldehyde and 10 molar excess of sodium cyanoborohydride were dissolved

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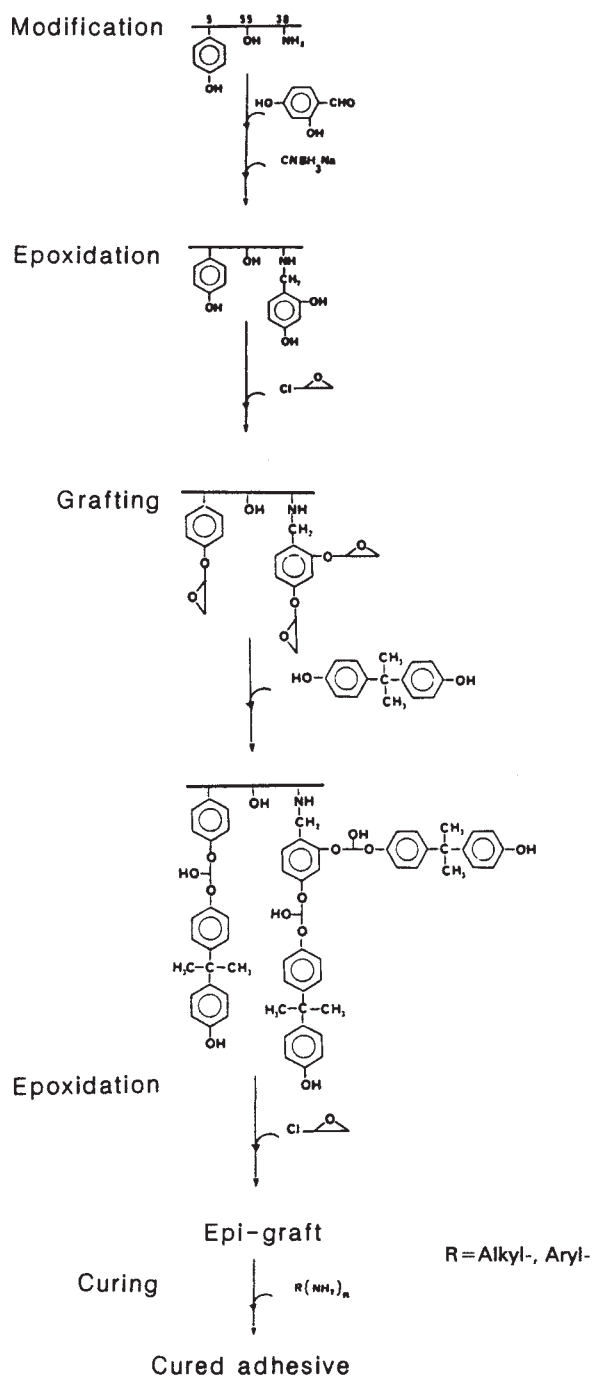


Fig. 1 The grafting procedure. The numbers along the top edge of the diagram indicate the number of similar chains in the molecule, where $\text{C}_6\text{H}_4\text{OH}$ is tyrosine, $-\text{NH}_2$ lysine and $-\text{OH}$ serine + threonine.

in 70 cm³ of DMSO and were added to gelatin solution, dropwise, under constant stirring and at room temperature for 30 min. The mixture was stirred for 5 h, the mixture was acidified with 6 M HCl to destroy unreacted cyanoborohydride and was precipitated by pouring into acetone. The precipitate was washed several times with acetone and was either dialysed against

0.006 M acetic acid or passed over Biogel P-2 column to remove unreacted low molecular weight reagent traces.

Epoxidation was effected when 3 g of 50% modified protein, 0.2 g of sodium hydroxide were dissolved in 25 cm³ distilled water, 2 cm³ of epichlorohydrin was dissolved in 5 cm³ of DMSO, in a three-necked flask equipped with a dropping funnel and condenser. The flask was heated to 60 °C and the protein solution was added dropwise for 15 min with constant stirring. After 5 h the mixture was poured into excess acetone, filtered, washed and dried *in vacuo*.

For grafting 8 g of epoxidized protein was dissolved in 50 cm³ DMSO in a three-necked flask, equipped with a condenser and a dropping funnel. The solution was heated to 50 °C and a 5 molar excess of bisphenol-A was added. After dissolution, equimolar amount of sodium hydroxide was added, dropwise, as a 40% aqueous solution. The reaction was run for 6 h and the product was obtained by pouring into excess acetone. The precipitate was filtered and washed with acetone several times. To remove the precipitated sodium hydroxide the product was dissolved in pH 7.2 borax buffer and passed over Biogel P-2 column. The product, which elutes within the void volume, was obtained by precipitation with acetone. This step was followed by a second epoxidation by dissolving the graft in distilled water and subjecting it to epoxidation exactly as discussed above.

Dry adhesive was cured by mixing it with a proprietary polyaziridine XAMA-2 (Cordoba Chemical Co.) into a homogeneous slurry and adding tetra-aminobenzene, pyromellitic anhydride and so on. The mixture was cured at 150 °C for 4 h and allowed to cool slowly to room temperature.

Curing can be achieved, as with any epoxide resin, in various ways: such as using aliphatic or aromatic polyamines, anhydrides, and Lewis acids. We have routinely used various polyamines and anhydrides, such as tetra-amino-benzene, diaminodiphenyl methane, diethylene triamine, pyromellitic anhydride, and nadic methyl anhydride. Up until the moment of curing, the graft is soluble in water as well as DMSO, ethyleneglycol, *N*-methylpyrrolidone.

The graft, prepared using resorcinol sulphide instead of bisphenol A, when placed between two aluminium coupons that have prepared with standard chromic acid/sulphuric acid mixture according to Forest Products Laboratory method and cured as described above, bonds the coupons with a tensile strength of ~4,000 p.s.i. (pounds per square inch). With steel coupons that have been prepared with sand-blasting only a tensile strength of 1,500 p.s.i. was achieved. Considering that the cured adhesive has <10% by weight epoxide resin grafted onto protein backbone, this represents a remarkable performance for a protein-based adhesive. Furthermore, this tensile strength was achieved with only 9% cross-linking density because there are only 38 lysine and hydroxylysines in the collagen molecule of which only 60% are modified and 50 aspartic and glutamic acids. Values ranging from 10 to 12–15 × 10³ p.s.i. have been quoted for the tensile strength of advanced epoxy—as well as phenolic based adhesive formulations. These adhesives, however, represent the ultimate result of studies that have spanned nearly 40 yr of work by innumerable workers. In these epoxide and phenolic adhesives, on the other hand cross-linking density is >50%. Thus, it is not unrealistic to expect that by developing selective modification methods for other modifiable amino acids the mechanical characteristics of this adhesive can only improve.

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Implications of ω -oxocarboxylic acids in the remote marine atmosphere for photo-oxidation of unsaturated fatty acids

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Specific organic compounds have been used as tracers of biological and anthropogenic source inputs to the marine atmosphere¹⁻⁸. This tracer approach uses the unique molecular signatures or fingerprints for various lipid compound classes of marine and terrestrial plants to identify the sources of the organic substances in aerosols⁹. However, many of these substances are not stable and undergo transformation reactions. For example, unsaturated fatty acids, which are major constituents of marine and terrestrial plants, have not been frequently detected in remote marine aerosols^{3,6,7}. The mechanism for the transformation of unsaturated fatty acids in the atmosphere has not been elucidated. In this paper, we report the discovery of a homologous series of ω -oxocarboxylic acids (C_4 - C_{14} ; C_9 being maximum) in marine aerosols. We propose that these compounds are oxidation products of unsaturated fatty acids in the marine environment. The discovery of ω -oxoacids, along with additional aerosol, sea water, and rain data for mono- and di-carboxylic acids, has led us to postulate a photo-induced oxidative reaction scheme for unsaturated fatty acids in the marine atmosphere and surface seawater.

Aerosol samples (2,500-6,800 m³) were collected with either Gelman-type A/E glass fibre or Pallflex-type quartz fibre filters using a Hi-vol air sampler¹⁰. Samples were collected from (1) a 20 m tower built on Enewetak Atoll 1, Marshall Islands⁶, (2) an 8 m tower on the RV *Moana Wave* in the North Pacific Ocean (~40° N, 170° W) and (3) 1 m above ground level in a heavily vegetated area of American Samoa. Blank filters were exposed to the air in the sample shelters during sample collection. Rainwater samples (1.30 litre) were collected on the tower at Enewetak¹⁰; a surface microlayer screen sample (2.95 litre) was collected in the upwelling area, ~50 km off the coast of Peru⁷.

A detailed account of the analytical methodology is given elsewhere¹¹. Briefly, half cuts of the filters were extracted with 0.1 M KOH in methanol (MeOH) under reflux. The extracts were divided into neutral and acidic fractions. The latter was treated with 14% BF₃/MeOH which derivatized the ω -oxocarboxylic acids to their corresponding ω,ω -dimethoxycarboxylic acid methyl esters (acetal esters). The esters were then separated by silica gel (1% H₂O, 200-400 mesh) chromatography by eluting with (1) hexane/CH₂Cl₂ (1:2) for monocarboxylic acid methyl esters, (2) CH₂Cl₂/Ethyl acetate (98:2) for dicarboxylic acid methyl esters and (3) CH₂Cl₂/MeOH (95:5) for acetal and hydroxyacid methyl esters. Rainwater and seawater microlayer samples were acidified (pH 1.5) with 6 M HCl and extracted with CH₂Cl₂ at the sampling sites. The extracts were later saponified with 0.5 M KOH in MeOH and separated into sub-fractions as stated above.

The subfractions were analysed by capillary gas chromatography (GC) on a fused silica DB-5 column (30 m $\times$ 0.32 mm). Gas chromatographic-mass spectrometric (GC-MS) analyses were conducted with a Carlo Erba 4160 GC interfaced to a Finnigan 4,500 mass spectrometer with an INCOS 2300 data system. The GC and GC-MS conditions are described elsewhere¹¹ and in Fig. 1. Methyl ester standards for structure determination and quantification were either bought or synthesized.

Figure 1 shows a reconstructed ion chromatogram and mass chromatogram at m/z 75 for the acetal and hydroxyacid ester fraction separated from a marine aerosol sample collected at Enewetak Atoll. A homologous series of C_4 - C_{12} ω -oxocarboxylic acids, C_9 being the most abundant, was detected in the

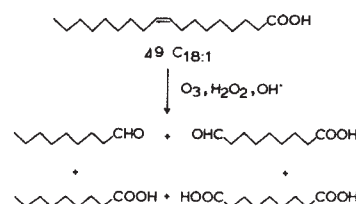


Fig. 1 Proposed pathway for the photo-oxidative production of ω -oxocarboxylic acids.

sample. Similar distributions were obtained for the other aerosol samples. The concentrations of ω -oxoacids are given in Table 1, as well as the concentrations of mono- and di-carboxylic acids. The ω -oxoacids were also detected in rainwater and sea water microlayer samples with a range of C_7 - C_{11} , C_9 being the dominant species in both samples.

To our knowledge, ω -oxoacids have not been reported in organisms. Hence, their presence in aerosol and seawater samples is most probably due to oxidative reactions. The predominance of the C_9 species suggests that biogenic unsaturated fatty acids are likely sources for the ω -oxoacids. Because plant-derived unsaturated fatty acids, such as oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids, generally contain double bonds at the C_9 position¹², oxidative cleavage of the double bonds produces predominantly the ω -oxononanoic acid. Indeed, laboratory oxidation of oleic acid with ozone has been shown to produce the C_9 ω -oxoacid^{13,14}. Thus, we propose that unsaturated fatty acids are most probably oxidized by O_3 , H_2O_2 or $OH^\bullet$ radical, resulting in aldehyde and carboxylic acid formation, as shown in Fig. 1.

This reaction scheme is further supported by the α,ω -dicarboxylic acid distributions found in the aerosol samples (Table 1) which also show a maximum at C_9 . A similar diacid distribution pattern has been reported in the solvent extractable fraction of Los Angeles rainwater¹⁵. Kawamura and Kaplan¹⁵ considered that the C_9 diacid was derived by oxidation of the $\Delta 9$ double bond of unsaturated fatty acids which were emitted from biogenic sources to the atmosphere. These results are consistent with the proposed reaction pathway (Fig. 1). It is of interest to contrast our reaction pathway to that proposed for the urban atmosphere: photo-oxidation of anthropogenic cyclic olefins may be the source of C_5 - C_7 ω -oxoacids (maximum at C_6)^{16,17} which have been reported in aerosol samples collected in Pasadena, California¹⁸.

The distribution of the ω -oxoacids in our aerosol samples suggests that the precursor unsaturated fatty acids should contain double bonds at different positions. It is most likely that phytoplankton and higher plants are the major sources of the $\Delta 9$ unsaturated fatty acids¹². However, bacteria contain positional isomers such as oleic ($\Delta 9$ $C_{18:1}$) and vaccenic ($\Delta 11$ $C_{18:1}$) acids¹⁹, thus monounsaturated fatty acids of bacterial origin could also contribute to the homologous series of ω -oxoacids. In fact, the C_{11} ω -oxoacid is relatively abundant in the marine aerosol samples (Fig. 2 and Table 1). Bacterial contribution to remote marine aerosols is also suggested by the presence of β -hydroxy fatty acids in the samples studied; these acids have previously been used as bacterial source indicators²⁰. Alternatively, the homologous series of ω -oxoacids could be derived from $\omega-1$ oxidation of the resultant ω -oxoacids during oxidative reactions or elimination of one methylene unit from the reaction intermediates¹⁷. For example, under certain oxidative conditions, both C_8 and C_9 products arise from the oxidation of the $\Delta 9$ $C_{18:1}$ fatty acid²¹.

There are three environments in which unsaturated acids could be photochemically oxidized to ω -oxoacids; on the glass fibre filter during sample collection; in the atmosphere; or in surface sea water. Unsaturated fatty acids were also detected in

Table 1 Concentrations (ng m⁻³) of monocarboxylic acids (Mono-), α,ω -dicarboxylic acids (Di-) and ω -oxocarboxylic acids (ω -Oxo-) in the remote aerosol samples

Carbon number	Enewetak Atoll (tower)			American Samoa (ground level)									North Pacific (ocean)		
	ENAS 14			SMAS 3			SMAS 22			SMAS 31			NPAS 27		
	5/15-19/79, 4,450 m ³	Di-	ω -Oxo-	1/24-25/81, 3,400 m ³	Di-	ω -Oxo-	7/10-12/81, 2,530 m ³	Di-	ω -Oxo-	8/1-5/81, 6,830 m ³	Di-	ω -Oxo-	5/25-29/86, 5,370 m ³	Di-	ω -Oxo-
C ₄	—	—	—	—	—	—	—	—	0.1	—	—	0.02	—	—	—
C ₅	—	—	—	—	—	—	—	—	0.09	—	—	0.02	—	0.010	0.011
C ₆	—	—	0.010	—	—	0.6	—	—	0.14	—	—	0.03	—	0.039	0.014
C ₇	—	—	0.012	—	—	4.6	—	—	0.35	—	—	0.10	—	0.10	0.062
C ₈	—	0.031	0.019	3.3	2.8	8.5	0.9	0.2	0.56	—	0.06	0.17	0.018	0.22	0.11
C ₉	0.060	0.070	0.030	10.9	22.5	44	3.5	0.71	0.91	0.33	0.14	0.27	0.031	0.46	0.17
C ₁₀	0.11	0.027	0.011	3.7	1.4	1.7	3.1	0.10	0.14	0.49	0.04	0.04	0.025	0.067	0.054
C ₁₁	0.029	0.026	0.024	0.9	1.3	1.6	1.1	0.12	0.37	0.04	0.05	—	0.019	0.14	0.088
C ₁₂	0.096	0.017	—	19	0.3	1.0	6.7	0.07	—	2.5	0.03	—	0.049	0.057	0.032
C ₁₃	0.043	0.012	—	0.6	—	—	0.7	0.06	—	0.33	0.02	—	0.016	0.045	0.025
C ₁₄	0.57	0.010	—	16	—	—	6.0	—	—	2.9	0.05	—	0.29	0.024	0.014
C _{16:1}	0.044	—	—	5.2	—	—	0.40	—	—	0.26	—	—	0.043	—	—
C _{16:0}	0.64	—	—	180	—	—	14	—	—	5.9	—	—	0.82	—	—
C _{18:2}	b.b.	—	—	44	—	—	2.6	—	—	0.43	—	—	b.b.	—	—
C _{18:1}	b.b.	—	—	190	—	—	3.6	—	—	2.1	—	—	0.043	—	—
C _{18:0}	0.083	—	—	69	—	—	5.4	—	—	1.8	—	—	0.21	—	—

b.b., Below blank; —, not detected. Concentrations of ω -oxoacids are presented as acetal methyl esters relative to methyl palmitate.

the filter samples (Table 1), so we cannot preclude the possibility that they are oxidized to ω -oxoacids on the filter. However, the finding of ω -oxoacids in significant concentrations in rainwater (31 ng l⁻¹) indicates that a substantial portion of the ω -oxoacids is present in the atmosphere. This suggests that photo-oxidation of unsaturated fatty acids on filters is not a major process.

Rather, ω -oxocarboxylic acids are probably produced in the atmosphere. Olefins in the atmosphere are known to exhibit significant reactivity toward ozone as well as toward hydroxyl radical²². For example, ethylene reacts with these oxidants to produce formaldehyde and formic acid^{22,23}. By analogy with olefins, double bonds of fatty acids can be oxidized with O₃ and OH radical in the atmosphere resulting in either aldehydes, monocarboxylic acids, ω -oxocarboxylic acids, or α,ω -dicarboxylic acids. The detection of C₇–C₁₁ ω -oxoacids (C₉ is the most abundant species) in rainwater collected at Enewetak Atoll supports the hypothesis for an atmospheric source of the oxoacids.

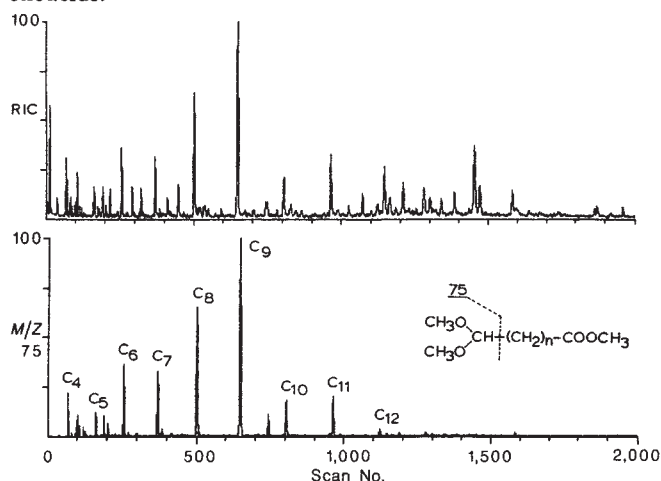


Fig. 2 a, Reconstructed ion chromatogram (RIC) and b, mass chromatogram (m/z 75) for the acetal and hydroxyacid ester fraction separated from aerosol sample (ENAS 14) collected at Enewetak Atoll, North Pacific. C_n=carbon numbers of ω -oxoacids. GC-MS conditions; injection: on-column injector, column: DB-5 fused silica capillary column (29.5 m $\times$ 0.32 mm), column temperature: 80 $^{\circ}$ C (3 min) programmed to 120 $^{\circ}$ C at 20 $^{\circ}$ C min⁻¹ and then to 305 $^{\circ}$ C at 4 $^{\circ}$ C min⁻¹, carrier gas: helium, ionization energy: 70 eV, scan range: m/z 50–500, scan interval: one second.

Surface seawater is also a likely environment for the formation of ω -oxoacids since surface active organic materials, including unsaturated fatty acids, are known to be enriched in the sea-surface microlayer^{24,25} where photochemically produced oxidants (such as H₂O₂, OH[•]) are also present²⁶. Indeed, upon analysis of a Peru seawater microlayer sample, we found the presence of C₇–C₁₁ ω -oxoacids, C₉ being predominant. The oxoacids produced on the sea surface are probably introduced into the atmosphere by sea-salt spray or bubble bursting mechanisms. It is of interest to note that normal C₆–C₁₀ aldehydes have been detected in sea water from this same Peru upwelling region²⁷. Gschwend *et al.*²⁷ considered that these aldehydes may be the oxidation products of unsaturated fatty acids, however their methodology did not allow detection of the other counterpart oxidation products (ω -oxoacids and diacids).

We conclude that ω -oxocarboxylic acids detected in aerosol samples from remote marine locations are key compounds in understanding photochemically-induced oxidative degradation of biogenic unsaturated fatty acids in the atmosphere and surface sea water. With the proposed mechanism (Fig. 2), we now have a better understanding of why unsaturated fatty acids are usually not detected in the marine atmosphere, in spite of their abundant presence in both marine and terrestrial plants. From the different sample types analysed, our results suggest that ω -oxoacids may be useful markers for evaluating the importance of photochemical reactions in the cycling of non-volatile unsaturated organic compounds in the atmosphere.

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Is the inner core of the Earth pure iron?

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The properties of the inner core (IC) of the Earth are widely assumed to be consistent with those of pure, solid iron in the hexagonal close-packed (ϵ) phase. This hypothesis is re-examined here using a density model of the Earth's core generated from extrapolated, static high-pressure data for ϵ iron and pyrite. Densities of constant-composition mixtures at room temperature and core pressures are compared with corresponding Earth-model densities. An effective volume thermal expansivity, α_{eff} , is calculated for a range of expected core temperatures that brings the room-temperature density into agreement with the Earth-model density at the pressure of the inner-core boundary (ICB). It seems that α_{eff} would have to be significantly larger than previous estimates of the thermal expansion at core conditions. A qualitatively similar conclusion is obtained if an isotherm reduced from shock-wave data is used for ϵ iron instead of the static data. We argue that, of several explanations for this difference (errors in Earth-model densities, a high-volume thermal expansivity at megabar pressures, a high-temperature core ($>7,000$ K), and the presence of a light component), the last alternative is the most probable and that the IC is not, therefore, pure iron.

The existence of an IC of the Earth was first proposed just over 50 years ago by Lehmann¹ to explain an apparent increase in the magnitude of the P-wave velocity deep in the core. Her interpretation was immediately confirmed by the travel-time studies of Jeffreys and Bullen² and Gutenberg and Richter³, and incorporated into their early Earth models. Bullen⁴ interpreted this increase of velocity as due to an increase in rigidity at the inner-core boundary (ICB), thereby distinguishing it from the Earth's fluid outer core. Birch⁵ had earlier suggested, from thermodynamic arguments, that the IC could be crystalline (mainly of iron composition). Further seismic evidence for the solidity of the IC has accumulated since then⁶, although the precise position of the boundary between the inner and outer cores appears to be uncertain⁷, and is determined by the gradient of rigidity with depth in the uppermost layers of the IC. It is generally accepted, however, that the Earth has a solid IC of radius 1,220–1,230 km (ref. 8) surrounded by a fluid outer core of radius 3,485 $\pm$ 3 km (ref. 9).

Separate to the question of the physical boundaries of core

regions are the inter-related, and contentious, questions concerning the composition and temperature profile of the core. The classical hypothesis as defined by Birch¹⁰, that the core is of predominantly iron composition, was originally proposed by analogy with the chemical composition of iron meteorites and the low moment of inertia-to-mass ratio of the Earth¹¹, well in advance of the first seismological evidence for the presence of a core. Birch¹⁰ showed that the outer core density determined from Earth models was lower than that expected for liquid iron in similar conditions—a result that has since been confirmed experimentally for iron and has resulted in an extensive geochemical debate on the nature of the predominant alloying element (that is, of atomic weight less than that of iron) in the outer core¹². The seismic properties of the IC, however, appeared to be in accord with those of pure, crystalline iron.

Birch's conclusions are now firmly established in geophysics¹³, and recently attention has been focused on whether the IC lies in the stability field of the ϵ or γ (face-centred cubic) phase of iron. Anderson^{14,15} has examined the likely position of the ϵ - γ -liquid triple point of iron (by extrapolation of the phase diagram of iron at lower pressures and temperatures) and concludes that the preferred phase is ϵ iron, which has also been favoured in other studies of the high-pressure data^{16–18}. We now re-examine the candidacy of ϵ iron as an IC phase in view of recent static data on the equation of state of iron¹⁹ and a critical comparison with the existing seismological Earth models.

Figure 1a shows quasihydrostatic, diamond-anvil cell compression data for ϵ iron and pyrite (FeS_2) together with third-order finite-strain extrapolations^{20,21} to core pressures. For pyrite, extrapolation is made from 40 to 328 GPa; for iron, the data extend to ~ 80 GPa and the extrapolation is, therefore, less extreme. Figure 1b shows a comparison between the extrapolated static isotherm for ϵ iron and the shock-wave isotherm of Brown and McQueen¹⁶. The difference between the two curves (0.4 Mg m^{-3} at the ICB) is about equal to the propagated uncertainty of the third-order fit to the static data.

Pyrite has been chosen for use in the present study because of the availability of new static data²² and because sulphur is considered a possible light component in the outer core²³. Other elements are also considered strong candidates and Ringwood²⁴ has proposed that oxygen is the major alloying component through incorporation of FeO in the outer core—an idea that has recently been given empirical support^{25–27}. Our conclusions do not depend on which light element is most abundant.

Core densities are calculated on the basis of a simple, ideal mixing model between ϵ Fe and FeS_2 . Figure 2a shows the density profiles calculated for the static data ranging from pure iron down to a mixture 60% iron, 40% pyrite throughout the pressure range of the core. Superposed on this plot is the PEM²⁸ pressure-density profile (solid bold curve) in the outer and inner core. No temperature correction has been made at this stage. The PEM profile was chosen for illustration and does not reflect a bias in the choice of seismic Earth model. In fact, there is no difference on the scale of the diagram between seismic models PREM²⁹, 1066(A or B)³⁰, or PEM in the outer core. Outer-core densities are well constrained on the assumption that Adams-Williamson conditions hold, that is, the outer core is neutrally stratified. These Earth models do not, however, resolve the density jump at the ICB precisely, and PEM, PREM, and 1066A,B require density jumps of 0.565, 0.597, and 0.868 Mg m^{-3} , respectively. Masters³¹ estimated the magnitude of this density jump to be $0.7(\pm 0.3\text{--}0.4) \text{ Mg m}^{-3}$ from an inversion of core-mode eigenfrequencies. This value may be revised downwards to $\sim 0.5 \text{ Mg m}^{-3}$ after a re-analysis of the revised normal mode dataset (G. Masters, personal communication). The magnitude of this density discontinuity determines the value of α_{eff} at the ICB calculated in our model.

For comparison with the static data, a second (hybrid) core model is constructed with the ϵ -iron isotherm derived from shock-wave (Hugoniot) data to 200 GPa (ref. 16) (Fig. 2b),

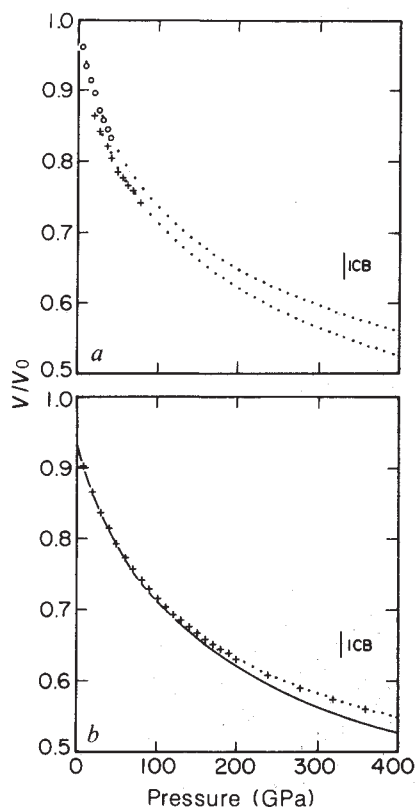


Fig. 1 Experimental compression data at room-temperature used to calculate core density. *a*, Static data on iron (+) (Argon medium) and pyrite (O) (Neon medium) extrapolated to core pressures with the coefficients of a normalized pressure versus strain fit obtained from a third-order, finite-strain analysis ($\bullet$)^{20,21}. Fits beyond third order are not justified by the data range. *b*, Comparison of (solid line) the static ϵ -iron isotherm with (+) the isotherm derived from shock-wave experiments. An extrapolation of the shock isotherm from 200 to 400 GPa (dotted curve) agrees well with points independently calculated (J. M. Brown, personal communication) from a shock equation of state (+). The deviation of the static and shock isotherms as a function of pressure should be considered an indication of the uncertainty in the density of ϵ iron at core pressures and room temperature resulting from the extrapolation.

combined with the static pyrite isotherm. Note that the iron isotherm derived from the Hugoniot data is compared purposely with the static result. In this way, the consistency between the two different experimental techniques can be checked (with some qualifications¹⁹) and, furthermore, no constraint can be placed on temperatures within the Earth through the assumption that they are equal to those calculated along the Hugoniot. Because the extrapolated ϵ -iron densities are slightly lower in the shock-wave experiment, the constant-composition curves are flatter (Fig. 2b).

The primary assumption to be tested is whether the IC is pure, solid ϵ iron. To bring PEM into exact agreement with the ϵ -Fe isotherm at the ICB, we have applied a positive, incremental density correction to the seismic Earth model, effectively lowering its temperature. The whole seismic profile is adjusted by this constant, positive increment in density (marked as $\Delta\rho_{\text{ICB}}$ in Fig. 2). For the static isotherm, the density of ϵ iron calculated at the ICB is 14.24 Mg m^{-3} and the density shift is $\sim 1.54 \text{ Mg m}^{-3}$ for the PEM model.

To determine whether all of this difference can be attributed to thermal expansion, estimates of core temperatures and the volume thermal expansion coefficient, $\alpha = 1/V(\partial V/\partial T)_P$, for ϵ iron at megabar pressures are required. Both of these quantities are poorly constrained. In the present model, the core tem-

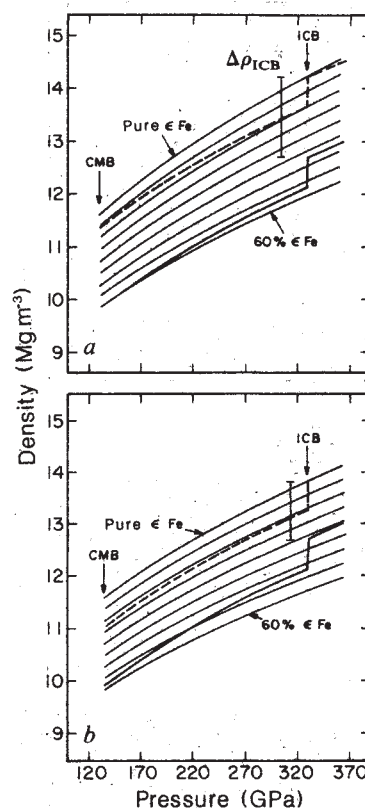


Fig. 2 Density profiles calculated for 100% iron down to 60% iron with 40% pyrite throughout the pressure range of the Earth's core for the static iron data (*a*) and the shock-wave isotherm (*b*). CMB marks the core/mantle boundary pressure and ICB the inner-core boundary. Superposed on these plots is the PEM density profile (solid, bold curve) in the outer and inner core. The density of a mixture containing a given fraction (by volume), x , of iron and $(1-x)$ of FeS_2 as a function of pressure is $\rho(P) = x\rho_{\epsilon\text{Fe}}(P) + (1-x)\rho_{\text{FeS}_2}(P)$. The bold, dashed curve represents the PEM profile shifted in density by an amount equal to the difference between the density of iron at the pressure of the ICB (328.87 GPa) and the density predicted by the Earth model in the inner core: $\Delta\rho_{\text{ICB}} = \rho_{\epsilon\text{Fe}}(P) - \rho_{\text{PEM}}(P)$, $P = P_{\text{ICB}}$.

perature has been varied between 2,000 and 8,000 K, and Fig. 3 shows the resulting trade-off between these temperatures at the ICB and the calculated value of the effective volume thermal expansivity, α_{eff} , as a function of the assumed composition of the (solid) IC: α is directly comparable to α_{eff} on the assumption that there is negligible dependence of α on temperature at constant pressure¹⁰. Table 1 lists the values of α_{eff} obtained with several Earth-model estimates of the density at the ICB for three probable values of the ICB temperature (4,000, 5,000, and 6,000 K) at pure ϵ iron composition. These temperatures can be readily accommodated by adiabats in the outer core and lower mantle, together with selected thermal boundary layers (say at D'' or 670 km) to match the surface temperature^{32,33}.

The important result of this analysis is that the value of α_{eff} needed to match experimental ϵ -iron densities with inner-core densities is significantly larger than previous estimates of α expected for core material. For example, on the basis of a thermodynamic model, Stacey³⁴ predicted values for α in the IC ranging from 6.9 to $7.2 \times 10^{-6} \text{ K}^{-1}$, and from 7.9 to $15.7 \times 10^{-6} \text{ K}^{-1}$ in the outer core. Values of α obtained in the present analysis are larger by about a factor of 5 at the ICB. From the shock-wave equation of state (EOS) for ϵ iron the values of α_{eff} are slightly reduced from the static EOS, but again are larger than previous estimates (Table 1). This result is dependent only on the compression data for ϵ iron and the assumption that the

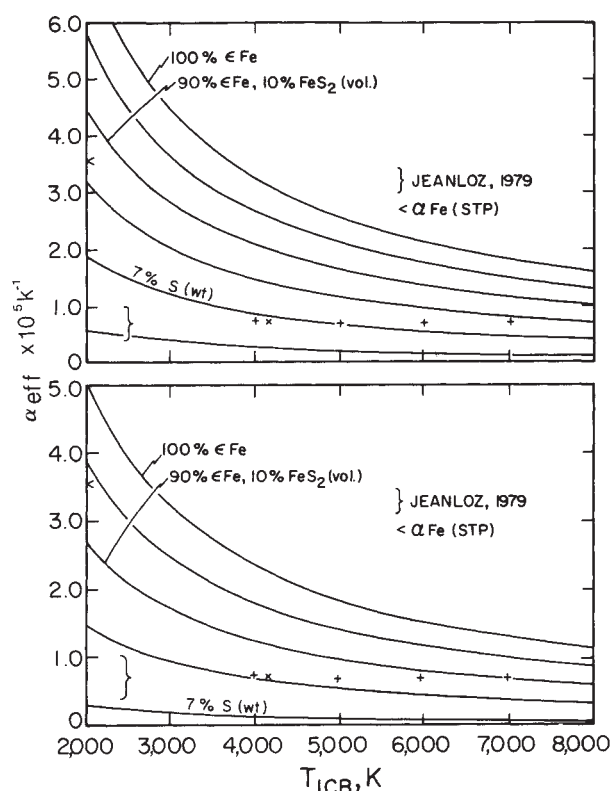


Fig. 3 Trade-off between core temperature and effective volume thermal expansivity of material at the ICB as a function of composition shown for (top) static and (lower panel) shock-wave data. The effective thermal expansion coefficient, α_{eff} is calculated according to the following relation

$$\frac{1}{(T_c - T_s)} \frac{\Delta \rho}{\bar{\rho}} = \frac{1}{(T_c - T_s)} \int_{T_s}^{T_c} \alpha dT = \alpha_{\text{eff}}$$

where T_s is the surface temperature of the Earth (300 K), and T_c is the temperature at the ICB. Estimates of volume thermal expansivity for core material from previous work are also shown ($\times$, ref. 34; $+$, ref. 16), together with the value for α iron at room conditions ($<$). The curly bracket represents the range of α estimated throughout the core from Hugoniot data to 150 GPa (ref. 18).

IC is ϵ iron. It does not depend, for example, on the assumption that pyrite is the light component.

Although the pressure dependence of the volume thermal expansivity is not yet well known for any material over the range of pressures within the Earth, it is generally argued¹⁰ that α should decrease with increasing pressure. Therefore α for iron in the core should be substantially less than the value at room pressure. The thermal expansion coefficient of ϵ iron has been measured up to 21 GPa, just above the $\alpha \rightarrow \epsilon$ transition, and does not differ significantly from the value for α iron³⁶. No measurements have been made close to core pressures, however. Band-structure calculations on iron at core pressures have provided estimates for the Grüneisen parameter, γ , of the γ -phase of iron³⁶, and the electronic specific heat, $C_{v,e}$, for the ϵ phase³⁷. It is not possible to estimate α by closure of the identity $\gamma = \alpha K_T / \rho C_v$ at pressure, because of the uncertainty in K_T the isothermal bulk modulus, and C_v the total (lattice plus electronic) heat capacity.

In view of the conclusion that the value of the thermal expansion needed to reconcile the ϵ -iron data with the Earth model IC densities is too large, the other assumptions of the model should be reconsidered. It is possible that core temperatures could be higher than previously thought, but with $\alpha = 7.2 \times 10^{-6} \text{ K}^{-1}$ (as suggested by Stacey³⁴) the core temperature required is $\sim 1.7 \times 10^4 \text{ K}$ which is improbably high. Another alternative is that the IC consists of γ Fe rather than

Table 1 Volume thermal expansivity, $\alpha_{\text{eff}} (\times 10^5 \text{ K}^{-1})$, for ϵ Iron at the ICB

T_{ICB} (K)	Earth model				
	PEM	PREM	1066	Other*	Shock†
4,000	3.28	3.14	2.54	2.98	2.36
5,000	2.58	2.47	2.00	2.35	1.86
6,000	2.13	2.04	1.65	1.93	1.53
4,168‡	0.72				

* Calculated with 0.7 Mg m^{-3} from ref. 31 added to PEM density at the outer core side of the ICB.

† Calculated from ref. 16 with PEM density.

‡ From ref. 34.

ϵ Fe. The expected density difference between these two phases¹⁶, however, is too small to account for the observed discrepancy. Although there is no substitute for actual data at core pressures, errors associated with the extrapolation procedure used in this study are considered small for several reasons: first, both the iron and pyrite data sets have been obtained with rare-gas solids as pressure-transmitting media, resulting in low scatter in the data and a well-constrained fit to the Birch stress-strain plot²⁰ for low order. (Systematic errors such as inaccuracy in the ruby pressure scale cannot be excluded.) Second, effects of high-pressure phase changes are not relevant to the arguments: for pyrite shock-wave results³⁸ suggest that there are none up to core pressures, and, for iron, the extrapolation involves only the ϵ phase. Finally, the finite-strain formalism used to extrapolate the isotherms could be in error. We consider the error to be small, however, because application of the method (with Eulerian strain) to the EOS of solid argon has shown that finite strain works well, at low orders, outside the range of the experimental data even for this very compressible solid³⁹.

The only remaining alternative appears to be that the inner core (as well as the outer core) contains a significant fraction of light component. In the present study, we estimate that 10–20% pyrite by volume would be required which is equivalent to a 3–7 wt.% sulphur. This density deficit could also result from the incorporation of oxygen into the IC⁴⁰.

The incorporation of light components into the IC has a direct bearing on the thermal regime of the core and the lower mantle, and on the driving mechanism for the geodynamo. The two most likely power sources for the dynamo are thermal convection resulting from heat loss at the core/mantle boundary (CMB) and compositional convection arising from fractional crystallization at the ICB^{17,41,42}. The thermal convection model requires a substantial ($\leq 10^3 \text{ K}$) temperature drop from the outer core to the mantle and, hence, a relatively high CMB temperature. An important geodynamical consequence is high heat flux and low viscosity in the D'' layer above the CMB which would then provide the environment necessary for formation of mantle plumes. Compositional convection would operate most efficiently if the IC is pure iron crystallizing from a eutectic composition alloy that would have formed at relatively low core temperatures. Under these conditions, little or no thermal boundary layer would be present above the CMB. Although our conclusion that light components are likely to be present in the IC does not rule out a contribution to the energy balance from fractional crystallization, it is more consistent with the high-temperature, thermal convection model for the outer core.

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Table 1 He isotope abundances and ($^3\text{He}/^{21}\text{Ne}$)_c ratios in Maui M85-5 olivine and clinopyroxene phenocrysts

	Olivine	Clinopyroxene
Vacuum crushing (no. 1; ~2 g)		
R/R_A	8.41	7.93
$^3\text{He}/^4\text{He}$	1.18×10^{-5}	1.11×10^{-5}
^4He ($10^{-9} \text{ cm}^3 \text{ g}^{-1}$)	13.9	21.0
Fusion of crushed powder (~0.6 g)		
R/R_A	692	133
$^3\text{He}/^4\text{He}$	9.69×10^{-4}	1.86×10^{-4}
^4He ($10^{-9} \text{ cm}^3 \text{ g}^{-1}$)	1.51	7.38
Total phenocryst		
R/R_A	75.4	40.5
$^3\text{He}/^4\text{He}$	1.06×10^{-4}	5.67×10^{-5}
^4He ($10^{-9} \text{ cm}^3 \text{ g}^{-1}$)	15.4	28.4
Vacuum Crushing (no. 2; ~4 g)		
R/R_A	8.42	7.75
$^3\text{He}/^4\text{He}$	1.18×10^{-5}	1.09×10^{-5}
^4He ($10^{-9} \text{ cm}^3 \text{ g}^{-1}$)	18.1	11.7
Cosmic-ray components		
$^3\text{He}_c$ ($10^{-12} \text{ cm}^3 \text{ g}^{-1}$)	1.45	1.30
($^3\text{He}/^{21}\text{Ne}$) _c	2.1	3.4

This table lists the measured He isotope ratios and ^4He contents extracted by vacuum crushing followed by fusion (in extraction no. 1); powder from vacuum crushing no. 2 was used for the fusion-extraction of Ne (Table 2). R/R_A is the $^3\text{He}/^4\text{He}$ ratio relative to atmospheric He ($R_A = 1.40 \times 10^{-6}$). Helium extracted by crushing is mantle-derived He characterized by $R/R_A = 8 \pm 1$ as in ocean-ridge basalts¹; this He component is trapped in fluid inclusions. The ^3He from fusion is corrected for the small amount of mantle He left in the powder by using the ratio in He from crushing and assuming all the ^4He is from the mantle: thus (^3He)_c = [$R_{\text{fusion}} - R_{\text{crush}}$] $\times$ (^4He)_{fusion}. The ($^3\text{He}/^{21}\text{Ne}$)_c ratios are based on the (^{21}Ne)_c data in Table 2. Precision estimates for the isotopic ratios and concentrations are 3% and 5% respectively.

Cosmic-ray-produced neon and helium in the summit lavas of Maui

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A ^3He component attributed to cosmic-ray-induced spallation reactions ($^3\text{He}_c$) in silicate minerals has recently been identified in lava flows on the summit of Haleakala volcano on Maui¹⁻³. This $^3\text{He}_c$ is observed in the helium extracted by fusion of olivine and pyroxene phenocrysts after first removing almost all of the mantle-derived helium by vacuum-crushing, a procedure which releases the volatiles trapped in fluid inclusions within the phenocrysts. Cosmic-ray-produced ^3He was found in the high-altitude (~3,000 m) summit lavas, which are ~500,000 years old, but was not observed in sub-surface samples which were overlain by younger basalts² or in a 200-yr-old lava flow at sea level on Maui^{1,2}. We report here the identification of cosmic-ray-produced $^{21}\text{Ne}_c$, in addition to $^3\text{He}_c$, in gases extracted by fusion of olivines and clinopyroxenes after vacuum-crushing. The observed ($^3\text{He}/^{21}\text{Ne}$)_c ratios and the ratio of $^{21}\text{Ne}_c$ in olivine to that in clinopyroxene are consistent with an *in situ* origin of $^3\text{He}_c$ and $^{21}\text{Ne}_c$ by cosmic-ray spallation reactions. This component could be important for interpreting helium isotopic data in terrestrial reservoirs. Geophysical applications could include determinations of erosion rates and exposure histories of terrestrial rocks.

The measurements of cosmogenic Ne and He reported here were made on gases extracted from Maui sample M85-5, a porphyritic alkali olivine basalt flow in the Kula formation, mapped as flow unit 'kp' by Macdonald⁴. The sample was collected by us from the surface of a flow close to the Haleakala Highway at an altitude of 2,780 m, in the lavas exposed ~0.8 km along the road before the turnoff to Kalahaku Overlook, and

~2.6 km NNE of White Hill (Pakaoao), where the samples were collected in which cosmogenic ^3He was found¹. The outcrop is outside the crater just below the rim, and represents a different flow unit of about the same age in the Pleistocene Kula formation⁴. Helium extractions in both vacuum-crushing and fusion methods and mass-spectrometric analyses were carried out as described¹; the Ne extractions were made on the vacuum-crushed powders in a new extraction system of low-Ne blank, which is on-line with a 60° 6-inch rare-gas mass spectrometer.

Tables 1 and 2 list the He and Ne measurements for sample M85-5. Two sets of vacuum-crushing extractions and analyses were made on both the olivine and clinopyroxene phenocrysts; the crushed powders from one set were fused for the He isotope analyses as reported previously^{1,3}. The second set was used for Ne work and replicate analyses of the He obtained by vacuum-crushing. Table 1 shows the He data obtained from both sets, which produced somewhat different amounts of helium but with identical $^3\text{He}/^4\text{He}$ ratios. About 10% of the He in olivine and 26% of the He in pyroxene remained in the powders after crushing, to be extracted by vacuum fusion. The $^3\text{He}/^4\text{He}$ ratios of the total phenocrysts are 75 times the atmospheric ratio for the olivine and 40 times for the clinopyroxene. In this lava, the concentration of cosmic-ray-produced $^3\text{He}_c$ is greater in the olivine phase, $1.45 \times 10^{-12} \text{ cm}^3 \text{ g}^{-1}$ (all volume measurements quoted at standard temperature and pressure) than in the pyroxene ($1.30 \times 10^{-12} \text{ cm}^3 \text{ g}^{-1}$), in eight other Maui lava flows studied recently this ratio was very close to unity³. We note that 80% of the ^3He in clinopyroxene, and 90% of the ^3He in olivine, is cosmic-ray-produced, so that the corrections for mantle ^3He (Table 1) are not very important.

Preliminary measurements revealed ^{21}Ne concentrations of only a few million atoms g^{-1} in the vacuum-crushed samples. As a consequence, Ne blanks were reduced using a new quartz double-vacuum extraction system; two-step extractions at 750

Table 2 Ne isotopes in Maui sample M85-5 olivine and clinopyroxene phenocrysts

		^{21}Ne	$^{21}\text{Ne}/^{20}\text{Ne}$	$^{22}\text{Ne}/^{20}\text{Ne}$	$^{21}\text{Ne}_c$
Olivine-1 (0.35 g)	750 °C	0.98 ± 0.09	0.0026 ± 0.0009	0.106 ± 0.015	0
	1,500 °C	3.43 ± 0.30	0.00585 ± 0.00032	0.103 ± 0.009	0.65 ± 0.09
Olivine-2 (1.02 g)	750 °C	0.53 ± 0.05	0.00277 ± 0.00025	0.095 ± 0.009	0
	1,500 °C	2.45 ± 0.25	0.01250 ± 0.00038	0.118 ± 0.009	0.71 ± 0.08
Clinopyroxene (0.73 g)	750 °C	0.88 ± 0.08	0.0032 ± 0.0005	0.104 ± 0.008	0
	1,500 °C	1.83 ± 0.18	0.00661 ± 0.00020	0.101 ± 0.008	0.38 ± 0.04
Atmospheric neon ⁶		—	0.00296	0.1020	—

All data were obtained on the vacuum-crushed sample listed as no. 2 in Table 1. Concentrations are given in $10^{-12} \text{ cm}^3 \text{ g}^{-1}$ and uncertainties are 1σ estimates.

and 1,500 °C (melt step) and a 30-min. extraction and clean-up schedule were used. Interference corrections were applied for Ar^{2+} , $\text{C}_3\text{H}_6^{2+}$, CO_2^{2+} and $\text{H}_2^{18}\text{O}^{+}$. Because the abundances of some ions change with time, all interfering species were monitored. The double/single charge ratios were constant in the range of observed Ne partial pressures. Because the Baur-Signer GS-98 ion source has some fluorocarbon background, the possibility of an HF^{+} interference was checked by varying the H_2 partial pressure. Uncertainties in the corrections for interferences are significant sources of uncertainties in the Ne isotope ratios, as they amount to 15%, 0.5% and 60% for the isotopes 20, 21 and 22, respectively. Ne sensitivities and mass discrimination of the electron multiplier were determined by pipettes of Ne of atmospheric composition⁶.

$^{21}\text{Ne}_c$ concentrations were calculated using the atmospheric $^{21}\text{Ne}/^{22}\text{Ne}$ ratio for trapped Ne. We emphasize that this choice is not critical, because the $^{21}\text{Ne}_c$ components at 1500 °C exceed the trapped ^{21}Ne concentrations by a factor of 1–3. Significant excesses of $^{21}\text{Ne}_c$ are observed in all 1500 °C extractions of the M85-5 phenocrysts, but not in the 750 °C fractions. This indicates a good retention of Ne_c in vacuum-crushed samples of both olivine and clinopyroxene. The average concentration of $^{21}\text{Ne}_c$ (Table 2) is $6.9 \times 10^{-13} \text{ cm}^3 \text{ g}^{-1}$ in olivine and $3.8 \times 10^{-13} \text{ cm}^3 \text{ g}^{-1}$ in clinopyroxene.

The $^{21}\text{Ne}_c$ and target element abundances (measured by microprobe) in olivine (Mg 22.8, Si 14.2, Fe 4.5 at.%) and clinopyroxene (Na 0.6, Mg 6.9, Al 3.5, Si 17.1, Ca 8.5 at.%) are consistent with the following approximate rate equation for production (similar to that for meteoritic spallation data⁷, but

assuming equal production rates for Mg and Na and for Si and Al, and an order of magnitude less for Ca): $P_{21} = \kappa(2.5[\text{Mg} + \text{Na}] + [\text{Si} + \text{Al}] + 0.1[\text{Ca}])$, where the target elements are given in atomic abundances and κ is the target-independent rate coefficient of average effective production. Absolute production rates for the predominantly neutron-induced reactions are only approximately known at present. Furthermore, the evaluation of κ requires information on shielding and erosion rates. Estimates of the required cosmic-ray exposure time are ~ 0.05 – 0.1 Myr and are consistent with a lava age of ~ 0.5 Myr, coupled with erosion rates of 5–10 m Myr^{-1} (refs 1, 3). The observed $^3\text{He}_c$ and average $^{21}\text{Ne}_c$ contents in olivine (Table 1) correspond to a production ratio $(^3\text{He}/^{21}\text{Ne}_c)_c = 2.1$, which is quite similar to ratios of 2.4 and 2.9 observed in meteoritic olivines^{5,8}. The $^3\text{He}/^{21}\text{Ne}$ ratio in clinopyroxene is 3.4, considerably larger than in olivine, because of its lower ^{21}Ne concentration.

It is interesting to compare the ^{21}Ne data with the $^{22}\text{Ne}/^{21}\text{Ne}$ ratios. Cosmic-ray-produced Ne ratios observed in meteorites exhibit a range $(^{22}\text{Ne}/^{21}\text{Ne})_c = 1.10 \pm 0.15$ (except for rare minerals), and this range is shown in Fig. 1 as the expected shifts due to addition of spallation Ne to trapped Ne of atmospheric composition. Figure 1 shows that the Maui data are consistent with such mixtures within error limits, although the trapped $^{22}\text{Ne}/^{20}\text{Ne}$ ratio can only be constrained to within about 5% of the atmospheric value.

These results show that cosmic-ray-produced components are sometimes significant and cannot be ignored in the interpretation of He and Ne isotopic data in terrestrial reservoirs. The nature of He and Ne components in phenocrysts of basaltic lavas and their relations to components in their host magma need to be assessed in detail before conclusions about the composition of their sources can be drawn.

Ne isotopic systematics are expected to provide valuable records of the erosional and tectonic history of rocks. The work on Maui basalts¹ and on cosmic-ray-produced ^{10}Be and ^{26}Al in terrestrial quartz⁹ suggests the possible application of these nuclides to studies of continental weathering and erosion processes. The extra information from cosmic-ray-produced Ne should allow comprehensive studies of erosion and tectonic uplift of terrestrial rocks and of geological units such as volcanic flows. Spallation Ne components should prove to be more reliable than ^3He , because Ne is less prone to diffusion losses over geological timescales, and it is less affected by the variable isotopic signature of the trapped (for example, mantle) component.

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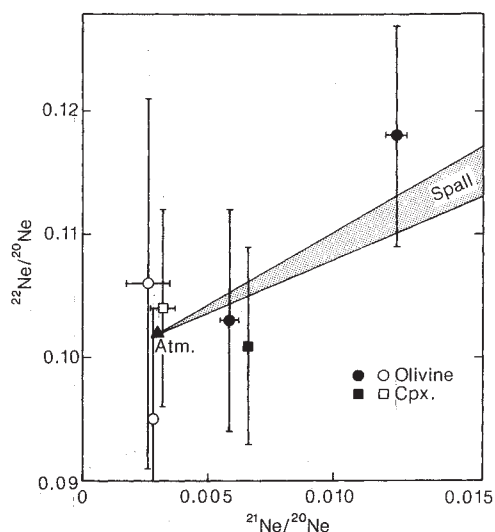


Fig. 1 Isotopic systematics of Ne extracted in vacuum-crushed M85-5 olivine and clinopyroxene. The shaded region represents the range of shifts expected from the addition of spallation Ne to trapped Ne of atmospheric composition. Open symbols are 750 °C fractions; filled symbols are 1,500 °C fractions; error bars correspond to 1σ uncertainties as given in Table 2.

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La–Ce dating of Lewisian granulites to constrain the ^{138}La β -decay half-life

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The isotope ^{138}La makes up 0.089% of natural lanthanum and exhibits a branched decay, by β^- to ^{138}Ce and electron capture to ^{138}Ba . The La–Ce isotope scheme is a potentially powerful adjunct to the Sm–Nd method for petrogenetic studies, but the best determination by counting of the β -decay half-life of La (3.02×10^{11} yr)¹ is in conflict with geological half-life determinations based on La–Ce and Sm–Nd mineral isochrons^{2–4}. To test these alternatives, I determined a six-point La–Ce isochron on whole-rock samples of Lewisian granulite-facies Archaean gneiss from NW Scotland. Here I report, based on a half-life of 3.02×10^{11} yr, a La–Ce age of $2,990 \pm 400$ Myr (2σ), which falls very well within the error of a whole-rock Sm–Nd date on Lewisian granulites of $2,895 \pm 100$ Myr (2σ). This half-life (equivalent to a La β -decay constant of 2.30×10^{-12} yr⁻¹) is therefore recommended for geological application of the Ce-isotope method.

Physical determinations of the La decay constants are hampered by the very low abundance of the radioactive isotope, the long half-life, and the difficulty of making accurate measurements on low-energy particles. Thus early values, particularly for the β -decay branch, are variable. Nevertheless, a weighted mean of eleven β -decay half-lives measured from 1951 to 1981 yields a value of $2.95 \pm 0.15 \times 10^{11}$ yr (ref. 2). A further complication in these determinations is caused by the hygroscopic nature of La_2O_3 , the material normally used in the experiments. La oxide may transform to the hydroxide, with a weight gain of ~17%, or the carbonate, with a weight gain of ~2.5%. However, in ten of the determinations assessed by Tanaka and Masuda², volatile data were unavailable. Assuming that in these cases La oxide had been completely transformed into the hydroxide, they applied a uniform 17% downward correction to all half-life results except those of Sato and Hirose¹, yielding a new average value of $2.69 \pm 0.24 \times 10^{11}$ yr.

However, Sato and Hirose found that material marketed as La oxide contained a mixture of hydroxides and carbonates displaying a weight gain of only 13%, so an arbitrary 17% correction of the (already variable) older data cannot be considered reliable. In contrast, the half-life of $3.02 \pm 0.04 \times 10^{11}$ yr determined by Sato and Hirose¹ was based on very good reproducibility between eight different determinations of various stoichiometric compounds. More recently, Norman and Nelson^{5,6} also made measurements on anhydrous oxide samples and obtained half-lives for mixed $\text{La}_2\text{O}_3 + \text{KCl}$ material ($3.29 \pm 0.27 \times 10^{11}$ yr) and pure La_2O_3 material ($2.95 \pm 0.22 \times 10^{11}$ yr) which straddle the value obtained by Sato and Hirose¹.

Despite a large variation in absolute values, all counting determinations since 1970 (including that of Sato and Hirose)¹ yield ratios of the electron-capture/ β -decay half-lives near 0.51. This allows an additional determination of the β -decay half-life through the electron-capture branch. Nakai *et al.*⁷ made a geological determination of the latter, based on a comparison La–Ba and Sm–Nd mineral isochrons, and argued that it supported the electron-capture half-life obtained by Sato and Hirose

(1.56×10^{11} yr). This therefore provides additional support for their β -decay half-life.

Tanaka and Masuda^{2,3} and Shimizu *et al.*⁴ made geological determinations of the La β -decay half-life by comparing La–Ce and Sm–Nd isochron results in separated minerals from the Bushveld complex, an Amîtsoq gneiss and a Finnish pegmatite. These give half-lives of 2.24, 2.10 and 2.33×10^{11} yr respectively, which are within the error of the 'volatile-adjusted' average half-life of 2.69×10^{11} yr, but well outside the error of the counting determination by Sato and Hirose. Because the geological determinations were made on mineral samples, however, they are susceptible to open-system behaviour during thermal events. In this situation a further geological half-life determination is warranted, using whole-rock samples less susceptible to metamorphic resetting.

Archaean granulite-facies gneisses outcropping in the Assynt area of NW Scotland display a range in rock type from ultrabasic to granitic, and a correspondingly large range in chondrite-normalized Ce/Yb ratio from 2 to 300 (ref. 8). The large REE (rare-earth element) fractionations between different samples of this suite, and their old age, makes them a good subject for the comparison of La–Ce and Sm–Nd age systematics.

Hamilton *et al.*⁹ determined a whole-rock Sm–Nd age on eight granulite-facies gneisses from the Drumbeg area of Assynt and three amphibolite-facies gneisses from Rhiconich, to the north. All the samples defined a single isochron, yielding an age of $2,910 \pm 50$ Myr (2σ) after recalibration of the Sm/Nd (weight) to $^{147}\text{Sm}/^{144}\text{Nd}$ (atomic) conversion factor¹⁰. Exclusion of the three amphibolite facies gneisses modifies this age by 15 Myr to $2,895 \pm 100$ Myr (2σ), well within the error of the original measurement.

Doubts have been expressed recently¹¹ about the reliability of whole-rock Sm–Nd isochrons on basic magmas emplaced through old cratonic basement. In these circumstances crustal contamination can lead to initial $^{143}\text{Nd}/^{144}\text{Nd}$ isotopic variations which may correlate with Sm/Nd, yielding a pseudo-age greater than the true age of emplacement. However, this is unlikely in the Lewisian gneisses because their Pb isotope compositions, probably the most sensitive indicator of hidden basement¹², show no evidence of contamination by a >2.9 Gyr crustal component¹³.

Six samples from the suite of Assynt granulites analysed by Weaver and Tarney⁸ and Hamilton *et al.*⁹ were selected for La–Ce concentration and Ce isotope analysis (Table 1). Five of these were chosen because they define a coherent suite of chondrite-normalized LREE (light REE) patterns (Fig. 1), spanning the maximum range in La–Ce ratios, yet displaying evidence of derivation by fractionation of related magmatic lineages. The LREE profile of the other sample analysed (7H) is not coherent

Table 1 La–Ce data on Drumbeg granulites

Sample no.	$^{138}\text{Ce}/^{136}\text{Ce}$	2σ	No. of scans of mass spectrum	La (p.p.m.)	Ce (p.p.m.)	$^{138}\text{La}/^{136}\text{Ce}$
8X	1.32809	12	1,900	1.13 1.17	4.25 4.27 4.24	0.127
7N	1.32852	6	1,500	3.29	11.00 11.12	0.167
15Z	1.32883	8	1,900	7.84	17.6 17.7	0.209
7H	1.32896	7	3,100	58.6	120 119	0.231
18T	1.32906	6	3,800	18.3 19.4	36.7 36.9	0.242
16Y	1.32957	8	1,400	31.2	44.6	0.330
BCR				24.8 24.5 26.2	53.5 53.5 53.8	

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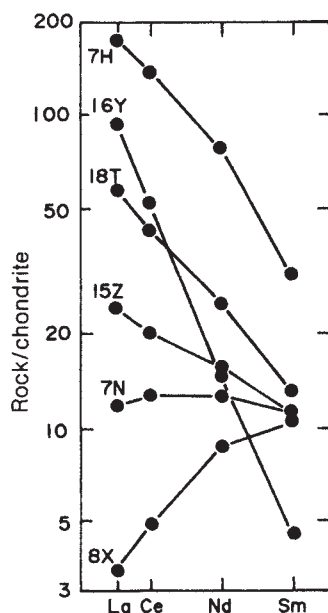


Fig. 1 Chondrite-normalized LREE diagram for Lewisian granulites analysed in this work.

with the remainder of the suite, but it was chosen for analysis because of its wider geochemical significance¹⁴. This sample lies near the centroid of the La-Ce isochron and is fully colinear with the compositions of the other samples, from which it may have been derived by secondary melting.

Ce isotope ratios, expressed as $^{138}\text{Ce}/^{136}\text{Ce}$ and normalized to $^{142}\text{Ce}/^{136}\text{Ce} = 58.14$, were measured in the oxide form on a Vg 54E mass spectrometer at East Kilbride. A detailed account of the analytical method is given by Dickin¹⁵. La and Ce concentration determinations were made by isotope dilution, using a mixed ^{142}Ce - ^{138}La spike. Ce isotope dilutions were analysed as the metal, but La was analysed as the oxide, as this avoids the need for a complete burn-off of Ba. Isotope dilution runs were not normalized for fractionation but an attempt was made to run samples under similar conditions to reduce its effect. La-Ce weight ratios are converted to $^{138}\text{La}/^{136}\text{Ce}$ atomic ratios by multiplying by 0.4715 (ref. 2).

Regression analysis of the La-Ce isochron data (Fig. 2) was done by least squares¹⁶. Errors in Ce isotope ratio were assigned individually to each data point, whereas errors in La-Ce ratio were estimated to be $\pm 1\%$ (1σ), based on the reproducibility achieved in duplicate isotope dilution determinations (which were then averaged). The regression calculation indicates a slight excess scatter over that predicted from estimated analytical errors (mean square weighted deviates (MSWD) = 2.28). However, on the relatively small sample set analysed there is a >95% probability that the scatter can be accounted for by analytical error without needing to invoke geological processes¹⁷. After expansion of analytical errors to encompass excess scatter (MSWD = 1), the regression yields a La-Ce age of $2,990 \pm 400$ Myr (2σ), using a half-life of 3.02×10^{11} yr, in excellent agreement with the Sm-Nd age. Exclusion of the two extreme points of the isochron, which generate the excess scatter, yields an almost identical age of $3,060 \pm 500$ Myr (2σ) with MSWD = 0.12. These results include the effect of electron-capture decay to ^{138}Ba on the abundance of the parent, although this causes a relatively small (15 Myr) reduction in calculated ages owing to the very long half-lives involved.

The initial $^{138}\text{Ce}/^{136}\text{Ce}$ ratios calculated for the six- and four-point isochrons respectively are 1.32735 ± 0.00020 and 1.32734 ± 0.00024 (2σ). Dickin¹⁵ calculated a present-day bulk Earth $^{138}\text{Ce}/^{136}\text{Ce}$ composition of 1.32857 from a correlation between $^{138}\text{Ce}/^{136}\text{Ce}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ in ocean island basalts. Using a chondritic La-Ce value of 0.380 (ref. 18) to calculate a bulk

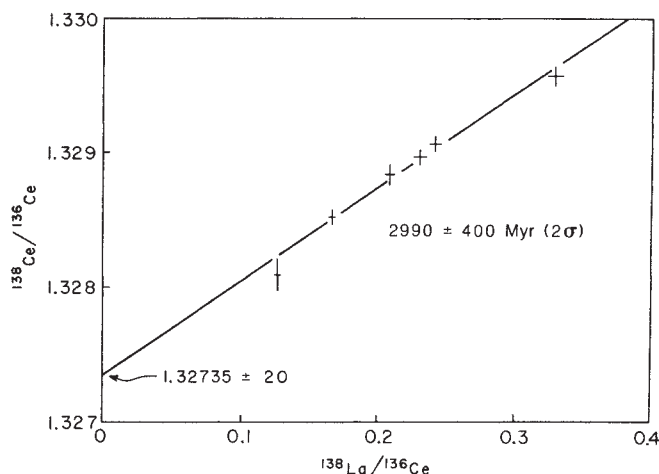


Fig. 2 La-Ce isochron diagram for Lewisian granulite-facies gneisses from Drumbeg.

Earth evolution line, the Lewisian gneiss initial ratio yields an ϵ_{Ce} of $+0.1 \pm 1.5$ (2σ), suggesting an approximately chondritic source. This is well within the error of the slightly depleted source indicated by the ϵ_{Nd} of $+2.0 \pm 0.9$ (2σ) for the Lewisian¹⁰.

Ce isotope data are a potentially powerful petrogenetic tool, because when combined with Nd isotope data on the same samples they allow modelling of the LREE profiles of components involved in mixing processes¹⁴. Because of the sensitivity of REE profiles to crystal-liquid distribution processes, this provides diagnostic information about the nature of the components, from which magmatic mixing mechanisms can be deduced. By improving constraints on the La β -decay half-life the present work prepares the way for the application of the Ce isotope technique to petrogenetic problems.

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Episodic growth of the Kodiak convergent margin

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Convergent plate boundaries have long been recognized as zones where deep-sea sediments become accreted to plate margins, causing the margin to grow with time¹. However, our understanding of the evolution of these margins is limited because accreted rocks are often too poorly exposed to unravel the complex history they

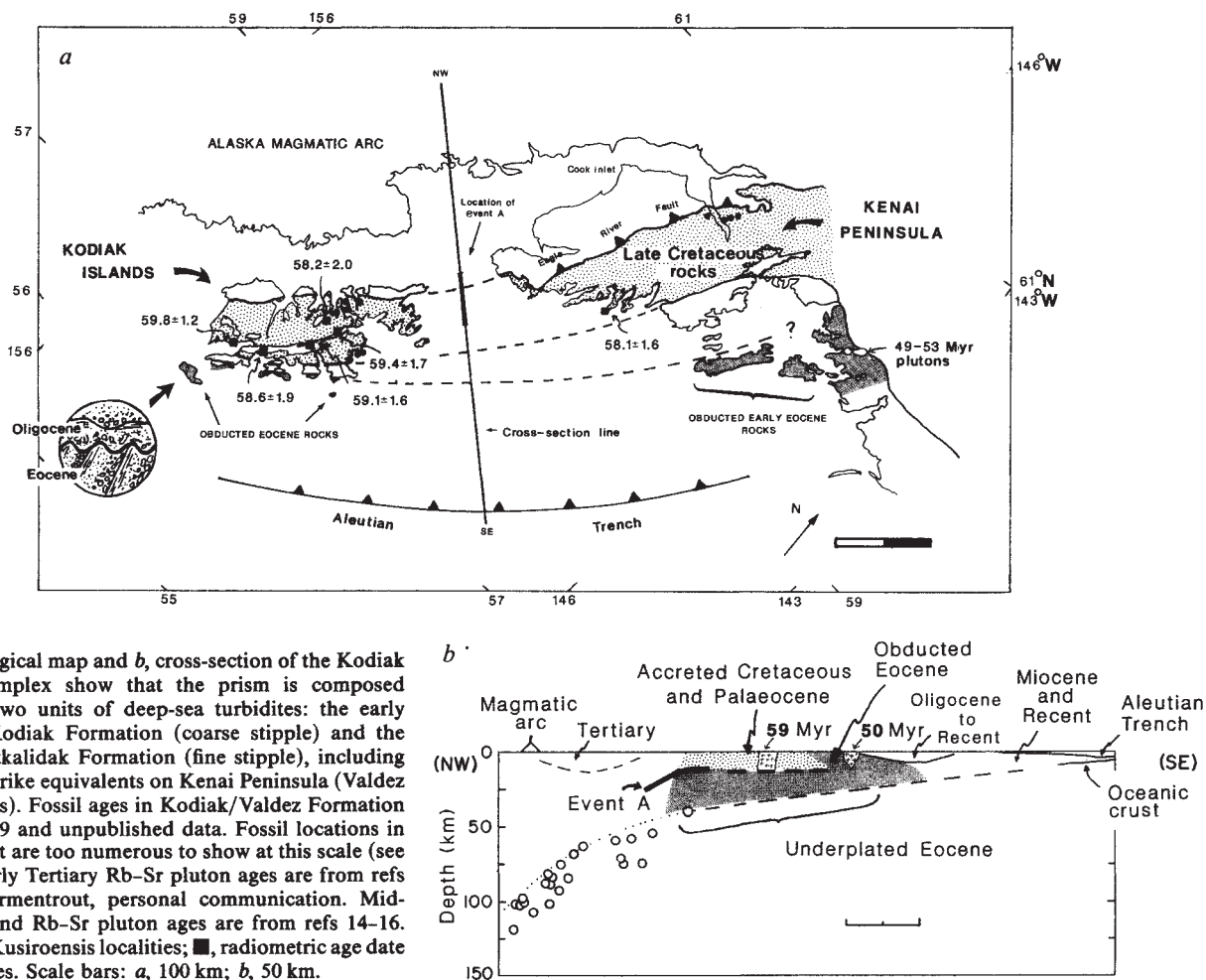


Fig. 1 *a*, Geological map and *b*, cross-section of the Kodiak accretionary complex show that the prism is composed dominantly of two units of deep-sea turbidites: the early Maastrichtian Kodiak Formation (coarse stipple) and the early Eocene Sitkalidak Formation (fine stipple), including possible along-strike equivalents on Kenai Peninsula (Valdez and Orca Groups). Fossil ages in Kodiak/Valdez Formation are from refs 8, 9 and unpublished data. Fossil locations in early Eocene belt are too numerous to show at this scale (see also ref. 14). Early Tertiary Rb-Sr pluton ages are from refs 7, 12 and J. Armentrout, personal communication. Mid-Tertiary K-Ar and Rb-Sr pluton ages are from refs 14-16. ●, *Inoceramus kusiroensis* localities; ■, radiometric age date localities. Scale bars: *a*, 100 km; *b*, 50 km.

record. Recent results from field, deep-sea drilling and high-resolution seismic-reflection studies indicate that various processes can occur in time along a specific margin². For example, both the middle America and Japan trenches are believed to record a history of strike-slip faulting and/or tectonic erosion before the periods of subduction accretion which presently characterize these margins^{3,4}. Here, we propose that growth of the Kodiak continental margin in south-west Alaska, one of the largest accretionary prisms in the world, has been dominated by two relatively short episodes of accretion; the first in the late Cretaceous and the second in the early Eocene. These two periods of growth appear to account for >70% of the margin growth during the past 75-100 Myr.

The evidence for episodic growth of the south-west Alaska margin comes mainly from two deep-sea turbidite units and two silicic plutonic sequences which cross-cut the turbidites. The turbidite units, the late Cretaceous Kodiak Formation and the Sitkalidak Formation (probably early Eocene), are beautifully exposed on the fjord-indented coastline of the Kodiak Islands, Alaska⁵. These islands expose a series of north-east-trending belts of accreted rocks that range from early Jurassic blueschists on the north-west to the weakly metamorphosed sandstone-shale deposits of the Sitkalidak Formation on the south-east. Most of the exposures on these islands, however, are composed of the deep-sea deposits of the Kodiak Formation (Fig. 1*a*). Moreover, recent geological data suggest that at depth the margin is composed dominantly of rocks probably equivalent to the Sitkalidak Formation⁶. Here we argue for the deposition, deformation and accretion of these two formations during two relatively short periods. We believe that together, these accretionary episodes resulted in the growth of the margin to nearly its present

volume, even though subduction and accretion seem to have been active along this margin for the past 75-100 Myr (ref. 7).

The Kodiak Formation contains a thick sequence of sandstone-shale turbidite deposits of early Maastrichtian (latest Cretaceous, 73-70 Myr) age. On Kodiak Island, six localities which span the across strike exposures of the formation have yielded fossils of the species *Inoceramus kusiroensis* (Fig. 1*a*)^{8,9}. This fossil appears to be tightly restricted in age. Jones and Clark⁹ believe *I. kusiroensis* was limited to the early Maastrichtian of the late Cretaceous period based on *I. kusiroensis*-bearing units on the Alaskan Peninsula to the north-west and in Japan and Russia. Similar *I. kusiroensis*-bearing deep-sea turbidites also occur along strike to the north-east on the Kenai Peninsula (Valdez Group, Fig. 1*a*) and to the south-west in the Shumagin Islands⁹. Thus, this sequence of sedimentary rocks is exposed for >1,500 km parallel to the modern Aleutian trench.

Detailed structural and petrographical studies of the Kodiak Formation indicate that the sediments of this formation were accreted to the margin before the emplacement of an earliest Tertiary quartz-dioritic batholith. We have found three important deformations in the Kodiak Formation. The first, D₁, and second, D₂, predate the emplacement of the batholith whereas the third, regional-scale folds and associated crenulation cleavage, may post-date the batholith and is not relevant here. D₁ is dominated by structural features that indicate layer-parallel extension and include extension veins, outcrop-scale normal faults and micro-scale normal faults. These structures are associated with cataclasis, the mechanical reorientation of phyllosilicates, and locally, with textures that indicate diagenetic reactions were still operative (for example, dissolved CaCO₃ veins, ref. 10,

and work in preparation). These observations suggest that D_1 occurred at relatively shallow structural levels of the subduction complex, consistent with the observation that this is the earliest deformation to affect the Kodiak Formation. We interpret the D_1 structures as indicating subduction of the Kodiak sediments beneath an older accretionary prism (work in preparation). D_1 structures seem to occur throughout the Kodiak Formation although they are overprinted by D_2 structures.

D_2 is characterized by a penetrative slaty cleavage and associated fold and thrust structures. The slaty cleavage is a distinctive feature of the Kodiak Formation, and it generally dips gently to steeply north-west, depending on structural level and position relative to the D_3 regional-scale folds. Regional metamorphism during D_2 is characterized by chlorite growth parallel to cleavage surfaces. Moreover, incremental strain studies indicate that the cleavage formed during a non-coaxial strain history consistent with the movement of thrust sheets along flats and over landward dipping ramps¹¹. Seaward verging folds, with an axial planar cleavage, and thrust faults are also common D_2 structures. The D_2 structures record the accretion by underplating of the Kodiak Formation sediments to the accretionary prism¹¹.

These D_1 and D_2 structures are metamorphosed by the emplacement of a quartz dioritic to tonalitic batholith that intrudes the Kodiak Formation (Fig. 1a). The metamorphic aureole contains the minerals cordierite, biotite, apatite and muscovite, all of which have grown over the slaty cleavage of D_1 . Radiometric dates of muscovite, biotite and potassium feldspar mineral separates from the batholith range from 56.7 to 60.9 Myr (ref. 12 and J. Armentrout, personal communication; note more dates are available than are shown in Fig. 1a). Therefore, the entire Kodiak Formation appears to have been deposited, deformed and accreted to the continental margin in the 14–15-Myr interval between the beginning of the Maastrichtian stage of the late Cretaceous (~73.0 Myr ago) and the emplacement of the Kodiak batholith.

The Sitkalidak Formation also consists of sandstone and shale turbidite deposits which are excellently exposed along the south-east side of the Kodiak Islands¹³. Possibly correlative rocks also occur along strike in Prince William Sound⁶. On Kodiak Island this formation has yielded one fossil of Eocene age. However, in Prince William Sound, structurally and lithologically similar rocks⁶ (Orca Group) contain abundant Eocene fossils¹⁴ and are intruded by post-accretion plutonic rocks that yield radiometric ages ranging from 49 to 53 Myr (Fig. 1a)^{15,16}. On Kodiak Island the Sitkalidak Formation is overlain by the Oligocene Sitkinak Formation along an angular unconformity (Fig. 1a)¹³. Thus, this formation and the possibly correlative units along strike seem to have been deposited and accreted to the south Alaska margin in the 4–7 Myr between the beginning of the early Eocene (57.0 Myr) and the time of pluton emplacement.

The style of offscraping of the Sitkalidak Formation is unique along the Kodiak margin because these rocks are characterized by landward-verging structures and a low grade of metamorphism (zeolite facies). Moore and Allwardt¹³ have interpreted the landward-verging structures as indicative of obductive offscraping (Fig. 1b) as opposed to the subductive offscraping and underplating which is typical of the rest of accretionary sequences exposed on Kodiak Island. They further argued that obductive offscraping resulted from the accretion of a thick, filled-trench sequence because landward-verging structures dominate the modern Washington convergent margin where a thick sequence of trench turbidites is being accreted.

It has recently been proposed⁶ that a volumetrically more significant accretionary event occurred contemporaneously with obductive offscraping of the Sitkalidak Formation. Based on geological and geophysical data, it was argued that part of the formation was underthrust (underplated) beneath the older, Cretaceous accretionary prism (Fig. 1b). The evidence for underplating comes primarily from: (1) the absence of landward tilting in the older accreted units; (2) structural indications of

vertical uplift; and (3) a deep (12 km) seismic reflector that is interpreted as the top of the underplated material (event A of Fig. 1a and b). This reflector provides important constraints on the volume of material that was underplated and it suggests that ~50% of the present accretionary prism is composed of rocks probably equivalent to the Eocene Sitkalidak Formation. The remaining, structurally higher parts of the present prism are composed mostly of the Cretaceous Kodiak Formation.

The Kodiak accretionary prism apparently grew to most of its present size during two relatively short periods of accretion: one in the latest Cretaceous and the other in the earliest Eocene. These results suggest that prism growth per volume, is episodic. Large volumes of sediment can be rapidly delivered to the accretionary prism either through high rates of subduction and trench sedimentation, or through both. Although the rates of subduction during the latest Cretaceous and the earliest Eocene along the Kodiak Islands are not constrained, the narrow range in ages of Kodiak and Sitkalidak formations suggests that high sedimentation rates were important for the accretion of large volumes of material. High sedimentation rates for the early Eocene are also indicated independently by uplift data from the Central Gneiss complex of British Columbia¹⁷. In this area, 30 km of uplift between 62 and 48 Myr ago could have produced a major portion of sediments needed for underplating along the Kodiak Shelf. Regional uplift in the latest Cretaceous is suggested by a post-Maastrichtian to early Tertiary angular unconformity on the Alaska Peninsula¹⁹ and uplift would have been a probable consequence of the collision of Wrangellia with North America at this time. Thus, rapid growth of the Kodiak accretionary prism may be indirectly related to collisions and mountain building events in nearby regions.

The dominant mechanism of accretion during these periods of rapid growth of the continental margin appears to be underthrusting or underplating of material beneath an older accretionary prism. The specific style of deformation within the underplated sequence is difficult to determine because these rock units are often deeply buried. However, structural studies in parts of the Kodiak Formation which are thought to have been underplated reveal fold and thrust geometries similar to duplex structures recognized in more classic foreland fold and thrust belts¹¹. These underplated sequences seem to have bypassed the zone of layer parallel shortening that is typical of frontal accretion²⁰.

Other convergent margins where relatively rapid offscraping and underplating may be important include the Makran region in southern Iran and Pakistan^{21,22}, the Oregon and Washington margin²³ and the southern Antilles²⁴. Along all these margins sedimentation rates are high and a thick sequence of the sediments occurs in the trench. Moreover, seismic reflection data from the Makran^{21,22} and the southern Antilles²³ indicate that almost half of the trench sediments are being thrust beneath the older accretionary prism and are bypassing the relatively shallow zone of deformation at the toe of accretionary prism. Thus, in the context of geological time, large accretionary prisms may form episodically and only when thick sediment piles are available for accretion. In addition, metamorphosed packages of turbidites may represent the remnants of underplated material because the structurally higher, offscraped packages would be the first to be eroded during uplift. Ancient examples of underplated material may include the structurally coherent blueschists of the Franciscan complex in California, the Jurassic slates and greywackes of Shikoku Island, south-east Japan, or the imbricated packages of deep-sea rocks in the southern uplands of Scotland.

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Bedform-generated convective transport in bottom sediment

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Convective flow within bed sediment is an important mechanism enhancing the mobility of chemicals, both natural and anthropogenic, and thermal energy in this region of aquatic environments^{1,2}. Experimental observations indicated that significant in-bed convection currents can be generated by water flowing over small obstructions on the surface of a porous bed. Significant porewater flow is induced by imbalances in pressure over distance, generated by differences in temperature, density and hydrostatic head³. We demonstrate here by laboratory simulation and a vignette model that flow over bedforms induces additional pressure imbalances which generate significant and complex convection currents within porous bed sediment. A model is proposed for estimating Peclet numbers for this effect. The results have particular application to chemical transport in the upper sediment layer that is often the recipient of high levels of chemical contamination. Although our analysis reflects river conditions, the results may have wider applications and include submarine currents moving over dune-like mega ripples on the ocean floor.

The flow of water over mobile, non-cohesive bed material such as silt, sand and gravel usually produces a series of sediment sandwaves⁴. These waveforms have been observed on the bottom of all aquatic environments where active water flow occurs over unconsolidated bed material. For this reason natural sandwave shapes were chosen as the bedforms for this study.

Natural streamflow processes were simulated in a flow channel. The channel was 2.4 m in length and 4.5 cm in width. The height was 40 cm with ~15 cm filled with a granular porous media, 15-cm water and 10-cm freeboard.

The stream-bed material was obtained from a Devon, UK stream and was partially classified to remove (~1%) the larger particles. The average particle diameter was 8 mm. This material was chosen for experimental convenience because the relatively high porewater velocities could be easily tracked and quantified with dyes. Water flow in alluvial channels moulds the bed material into several geometric forms or bed configurations. The common forms include ripples, dunes, or mega ripples and antidunes⁴. A series of two-dimensional wave forms, with a shallow upstream slope and steep downstream slope (Fig. 1), were created by hand to cover the entire bed surface of the channel. The large waves were 55 cm in length, λ , and 5 cm in height Λ ; the smaller waves were 25 cm in length and 2.5 cm in

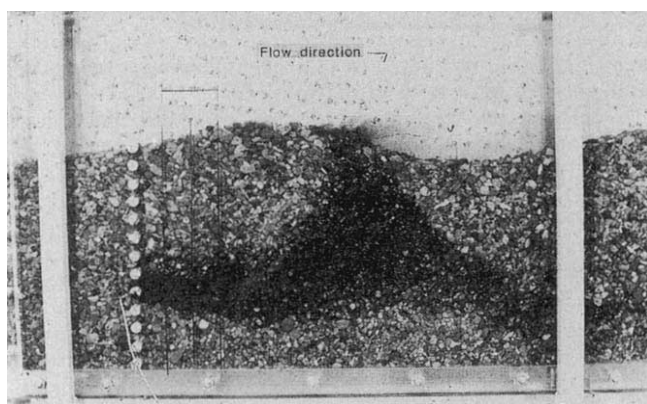


Fig. 1 Typical trace experiments showing suction on leeside of bottom obstruction drawing the dye upward to the bed surface. λ , 55 cm; Λ , 5 cm. Scale marker = 6 cm.

height. Length-to-height ratios, λ/Λ , and general wave shapes were as they are commonly found in nature⁴.

The sides and bottom of the flow channel were made of transparent Plexiglass. Holes were drilled into one side to serve as dye injection ports. Stainless-steel syringe tubing (1-mm diameter) conducted the dye solution to the injection point. Flow streamlines were mapped by pencil tracings directly onto transparent overlay paper. Porewater velocities were measured by timing the dye front movement over a known distance. Diluted nigrosine dye, purple/black in colour, was used in all quantitative measurements.

A typical dye-trace experiment is shown in Fig. 1; water flow is from left to right. An excessive dye injection rate was used in this particular experiment to enhance the photograph and highlight certain details. The injection port is the fifth position from the channel bottom. The dye trace moves from left to right and the plume increases in width with distance from the injection point. In general, water enters the waveform between the trough and the crest on the upstream face and exits between the crest and the trough valley on the downstream face. The first 10 cm of flow distance typifies dispersive processes in porous media. Beyond this point the entire plume bends upward towards the wave peak. A low-pressure zone exists on the leeside of the wave peak and draws the flow to that point. The plume width appears to decrease during its upward path and the dye leaves the bed and enters the free water. A dye cloud can be seen on the leeside of the peak. Within the bed the dye plume splits. Another portion of the dye plume is forced downward into the bed at ~45° angle with the horizontal. Part of the plume con-

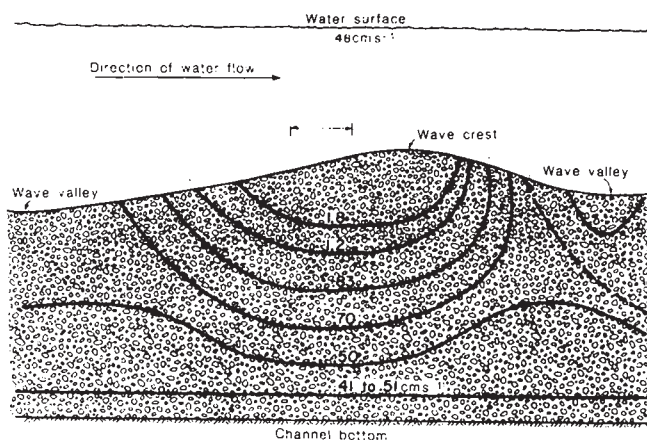


Fig. 2 Composite fluid dynamic diagram illustrating streamlines, in and out flow regions and magnitude of porewater velocities. λ , 55 cm; Λ , 5 cm. Scale marker = 6 cm.

tinues to move downstream. Fresh water enters the bed through the upstream face of the wave. This process is not shown in the trace (Fig. 1) but its effect can be seen. Inflowing water causes the downward movement of the split plume.

Seven dye-trace experiments were performed to obtain maps of porewater flow streamlines and velocities within the bed material (see Fig. 2); the general pattern of the in-bed, waveform-induced flow is illustrated. Changes in both size of the waveforms and the free-water velocity produce similar flow patterns (see Fig. 3). Streamlines, shown widely spaced, enter on the upstream side of the wave (see Fig. 2). Flow into the bed was directed at $\sim 45^\circ$ downward and to the right. Streamlines rotated counter-clockwise, converged (spacing decreased) and finally turned upwards before exiting from the bed through the downstream side of the wave. In-bed porewater velocities were highest through the wave-peak zone and decreased downward. Linear streamlines, running parallel to the free-water surface, were observed near the bottom of the bed. It appeared that the non-linear streamlines, which result from the waveform generated flow, merged with the linear streamlines at ~ 5 wave heights ($\sim 5\Delta$) below the wave crest. The average porewater (front) velocity in the bed is calculated to be 0.93 cm s^{-1} . The free-water surface velocity in the channel was 48 cm s^{-1} .

Streamline trajectories were drawn at the midpoint of the plume. On the longer trajectories, dispersive processes caused the plume to fade making the tracking process difficult. In these cases, several injection points were needed to track the streamline completely. In-bed porewater velocities were measured by timing the dye frontal movement between fixed positions. Coefficients of variation for frontal velocity measurements ranged from 0.076 to 0.27. Pure nigrosine is more dense than water, however, measurements indicate that the dye solution

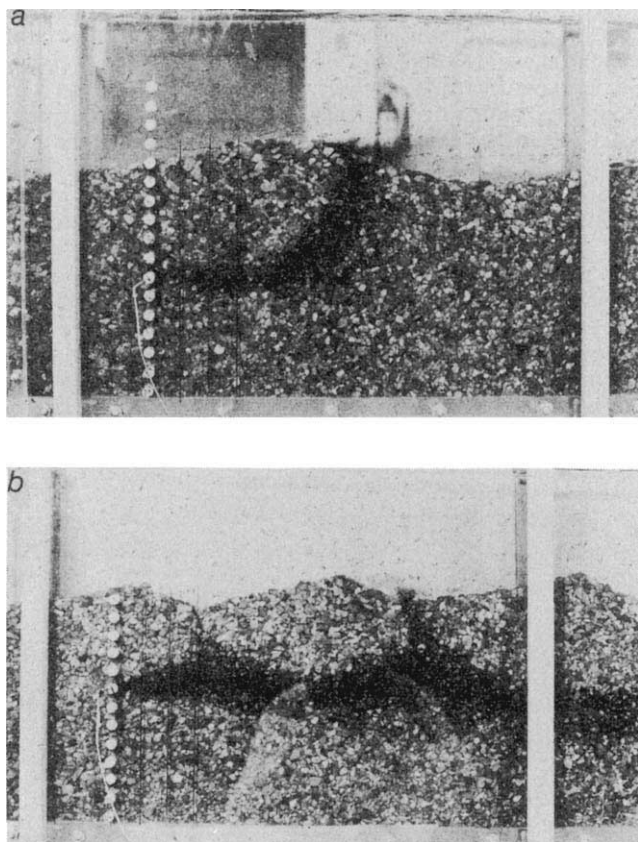


Fig. 3 a, Dye-trace experiment shows curved streamlines observed under wave crest. λ , 55 cm, Δ , 5 cm. (b), Dye trace experiment showing complex in-bed flow patterns generated by multiple surface waveforms. λ , 25 cm; Δ , 2.5 cm.

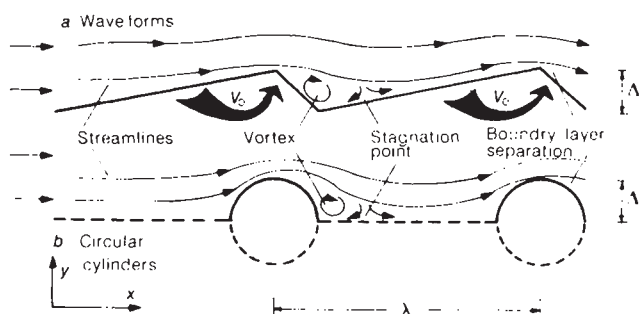


Fig. 4 Analogies of flow conditions around objects in a flow field. a, Over-bed and in-bed flow for a sandwave bedform. b, Flow across circular cylinders.

density does not introduce a significant natural convection component to the velocity measurement.

The 'wall effect' on the measurement of the porewater frontal velocity was of some concern. Dye movement could be observed only near the walls. This region is also one of lower particle packing density (that is, higher bed porosity). However, repeated dye injections at the wall and midway through the bed yielded statistically similar porewater frontal average velocities. Dye-trace studies were performed above the bed to establish the general pattern of the streamlines, the boundary layers separation point, the vortex region, and the position of the stagnation point. There is an obvious analogy between turbulent flow across cylinders and waveforms (see Fig. 4) and, therefore, a pressure drop must exist between the upstream face of the waveform and the vortex region on the downstream face⁵. The total pressure gradient in the bed zone is the sum of the wave contribution and the hydraulic gradient (slope of the water surface) contribution:

$$dp/dx = dp/dx|_w + dp/dx|_h \quad (1)$$

where p is the pressure and x is the distance downstream. The pressure drop across sand-coated two-dimensional triangular elements, simulating ripples and dunes, has been measured and reported as a pressure coefficient, c_p (ref. 6). This coefficient can be used to approximate the average convective velocity, V_0 , within the bed. Converting the pressure drop to a gradient for the triangular elements yields the approximation:

$$-dp/dx|_w = c_p \rho V^2 / \lambda \quad (2)$$

where V is the average stream velocity and ρ is the fluid density. The differential dx is approximated by $\lambda/2$. The stream surface hydraulic gradient can be approximated by:

$$-dp/dx|_h = \rho g s \quad (3)$$

where g is gravitational acceleration and s is the slope of the water surface.

D'Arcy's law is assumed to be applicable to the in-bed flow process:

$$V_0 = -\frac{K}{\mu} dp/dx \quad (4)$$

where V_0 is the superficial porewater velocity, K is the permeability of the porous bed material and μ is the porewater viscosity. Combining equations (1)–(4) yields:

$$V_0 = \frac{K}{\nu} (c_p V^2 / \lambda + g s) \quad (5)$$

where $\nu \equiv \mu/\rho$. Equation (5) provides a means of estimating the magnitude of the convective in-bed flow as a function of stream-flow and stream-bed parameters. The Peclet number for the bedform induced convection is defined by $Pe \equiv V_0 l / D$ with $l = 5\Delta$ and D the effective diffusion coefficient of the solute molecule or ion. Eliminating the stream-surface slope contribu-

tion from equation (5) yields V_0 for substitution into the Peclet number equation:

$$Pe = c_p 5 \Lambda KV^2 / \nu D \lambda \quad (6)$$

For the triangular element model, the average c_p for the waveform shape is $0.5 (\Lambda/h)^{3/8}$ where h is the water depth⁶.

The average porewater velocity under the crest (Fig. 2) is 0.93 cm s^{-1} . In experimental conditions: porosity = 0.44 (ref. 7); $K = 8.52 \times 10^{-5} \text{ cm}^2$ (ref. 7); $V = 48 \text{ cm s}^{-1}$; $s = 0.01$, $h = 15 \text{ cm}$; $\Lambda = 5 \text{ cm}$; $\lambda = 55 \text{ cm}$; $\nu = 0.009 \text{ cm}^2 \text{ s}^{-1}$, and $g = 981 \text{ cm s}^{-2}$. Equation (5) yields $V_0 = 0.24 \text{ cm s}^{-1}$. This underestimates the apparent observed value ($V_0 = 0.93 \times 0.44 = 0.41 \text{ cm s}^{-1}$) by a factor of 2. Considering the simplicity of the model and the complexity of the coupled fluid dynamic processes across the bed interface, this degree of discrepancy is not unexpected. Further experiments will allow refinement of the model through observations of c_p values which reflect a porous interface condition rather than the solid interface conditions inherent in the triangular element data.

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In conclusion, experimental observations indicated that significant in-bed convection currents can be generated by water flowing over small obstructions on the surface of a porous bed. A complex flow pattern was observed within the 'sand wave' shaped bedform to a depth 5 times the obstruction height. A model based on pressure drop across simple shapes coupled with D'Arcy's law provided the theoretical framework to yield an (preliminary) algorithm for predicting in-bed velocity (equation (5)) and Peclet number (equation (6)) based on stream and bed parameters. Measured values of the permeability and porosity for the Nile River sediment at Luxor⁸ and estimates of the flow⁹ and bedform dimensions: $K = 6.25 \times 10^{-9} \text{ cm}^2$, $\varepsilon = 0.419$, $V = 91 \text{ cm s}^{-1}$, $h = 600 \text{ cm}$, $\Lambda = 50 \text{ cm}$ and $s = 0$, yields $Pe = 620$. In this calculation, $D = 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ and $\nu = 0.009 \text{ cm}^2 \text{ s}^{-1}$. A similar Peclet number was obtained for the Mississippi River upper sediment layers. Because the calculations yield Pe values $\gg 1$, chemical transport by convection dominates that of diffusion by a significant margin.

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Perennial N_2 supersaturation in an Antarctic lake

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The dry valleys of southern Victoria Land in Antarctica contain several closed basins in which perennially ice-covered lakes are found. One of the most unusual features of these lakes is the occurrence of high O_2 concentrations in the water column; values ranging from slightly more than saturation to more than four times saturation have been reported^{1–3}. Recently, we considered a bulk O_2 budget for Lake Hoare, Antarctica, which led us to suggest that biological processes alone were not sufficient to explain the observed elevated oxygen levels³. Consequently, there must be a non-biological source of O_2 . We suggested that this source results from the exclusion of O_2 during the freezing of aerated meltstream water at the bottom of the ice cover, and predicted that this physical mechanism should also enhance the other atmospheric gases. Here we report the results of a study which, for the first time, documents the supersaturation of N_2 in a lake. Dissolved N_2 levels of 145% and 163% were determined from samples taken just below the ice cover and at a depth of 12 m, respectively. The relative importance of biological and abiological sources is reflected in the ratio of N_2 concentration to O_2 concentration. In Lake Hoare this ratio was 1.20 at the ice/water interface and 1.05 at 12 m; considerably different from the ratio in equilibrium with air (~1.8). Based on these results, we have determined that about half of the net O_2 production in the lake is the result of biological processes.

Lake Hoare (77°38' S, 162°53' E) is at the eastern end of Taylor Valley in southern Victoria Land, Antarctica. It is 58 m above sea level, 4.1 km long, 1.0 km wide, with a surface area of 1.8 km², a maximum depth of 34 m, and a mean depth of 14.2 m. The

perennial ice cover of Lake Hoare overlies water at a temperature of ~0 °C. Less than 1% of the incident photosynthetically active radiation (400–700 nm wavelength) penetrates the ice cover^{2,4}. The ice cover also prevents wind-generated mixing and greatly restricts exchange of gases with the atmosphere. The lack of mixing results in a perpetually stratified water column which is anoxic below 26 m. Along the margins of the ice cover is a region of annual ice which melts most summers, creating a moat ~5 m wide that is relatively well-mixed by frequent winds. The lake receives both water and sediment from glacial meltstreams and from nearby Lake Chad during the austral summer. Lacking outflowing streams, it loses its water by ablation and sublimation at the surface of the ice cover and evaporation from the moat.

We have developed a bulk O_2 budget³ which shows two primary net sources of O_2 : (1) a biological source resulting from the burial of organic carbon in the sediment which leaves a proportionate amount of O_2 in the water column; and (2) a non-biological, physical source resulting from the accumulation of gases which are carried into the lake by the aerated meltstream and forced into the water column when the water freezes to the bottom of the ice cover.

Dissolved gases enter Lake Hoare in glacial meltstreams and overflow from Lake Chad. In steady-state conditions incoming water is balanced by ice loss from the ice-covered surface. As new ice is formed at the bottom of the ice cover, the atmospheric gases dissolved in the water, including O_2 , N_2 , CO_2 , Ne and Ar, are forced from the ice back into the water^{5,6}. The effectiveness of this exclusion mechanism is dependent on the rate of gas bubble formation at the ice/water interface; which in turn depends on several factors including the rate of freezing and the particulate and gas content of the water⁵.

Meltwater input coupled with ice ablation output represents a net source of atmospheric gases into the lake³. In steady state, there must be loss mechanisms to balance the input of gases from the meltstream and from biological production. Bubble formation and loss of gases from the moat formed during part of the austral summer are probably the major loss mechanisms³.

The two principal atmospheric gases (N_2 , O_2) are both influenced by the non-biological processes affecting the gas balance of the lake water. Non-biological processes will act on N_2 and O_2 in a manner determined by their solubility in water, maintaining the ratio, N_2/O_2 , at that value characteristic of

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Table 1 Nitrogen (S_N) and oxygen (S_O) saturation and volume ratio

Sample	S_N	S_O	N_2/O_2 by volume
Meltstream	1.03 ± 0.05	0.96 ± 0.07	1.93 ± 0.05
Ice/water interface	1.45 ± 0.03	1.97 ± 0.03	1.20 ± 0.03
12 m	1.63 ± 0.16	2.55 ± 0.20	1.05 ± 0.10
Equilibrium values	1.00	1.00	1.8

The saturation is defined as the volume concentration of gas divided by the value that would be found in water in equilibrium with the atmosphere at a total pressure of moist air of 1 atm; $18.42 \text{ cm}^3 \text{ l}^{-1}$ for N_2 and $10.22 \text{ cm}^3 \text{ l}^{-1}$ for O_2 at 0°C (ref. 9).

water in equilibrium with the atmosphere (~ 1.8). However, biological processes will affect the O_2 concentrations to a much larger extent than they will affect N_2 , thus altering the N_2/O_2 ratio. Therefore, this ratio can be a useful 'signature' of biological and non-biological gas production.

In Lake Hoare, as in most ecosystems, the impact of biological activity on the total N_2 budget is negligible. Allnutt *et al.*⁷ failed to detect the fixation of N_2 by microorganisms in the below-ice habitats of Lake Hoare. However, fixed-nitrogen does enter the lake through the meltstream⁸, and total input of non- N_2 nitrogen into the water column is $\approx 11 \text{ kg-N yr}^{-1}$. This is negligible compared with the total dissolved N_2 entering through the meltstream which is $\sim 12,000 \text{ kg-N yr}^{-1}$. Hence, the efficacy of the abiotic processes can be tested by measuring the N_2 concentration in the lake water, and the ratio of N_2/O_2 saturation will reflect the relative importance of the biological to non-biological processes in shaping the gaseous environment in the lake. This is a unique and simple method for quantifying biological processes in this lake.

During the 1985–86 field season, water samples were collected from the Canada Glacier meltstream (five replicate samples) and within Lake Hoare (seven replicate samples from each location) at the water/ice interface (immediately below the perennial ice cover) and at 12 m depth. The lake samples were collected by a SCUBA diver from a dive hole which was $\sim 100 \text{ m}$ from the snout of Canada Glacier and 500 m from the inlet of the meltstream. Samples were obtained *in situ* by inserting a bubble-free, 50-cm³ syringe through a septum into an evacuated glass serum bottle to which 10 cm³, at 1 atm, of 1% Kr in He had been previously added to assess any possible leakage. The bottles were filled to capacity and kept in the dark until analysis. The head space gas overlying the water sample from each sample was analysed by gas chromatography. This was accomplished by expanding the headspace gas into a vacuum line and a six-port Valco valve equipped with a 5-cm³ injection loop and injecting samples of the headspace gas repeatedly onto a 2 m $\times$ 0.31 cm 50/60 mesh molecular sieve column. The column was operated at 0°C with He as the carrier gas flowing at a rate of $15 \text{ cm}^3 \text{ min}^{-1}$. A Carle model 1100 thermal conductivity microdetector and a Hewlett-Packard 3390A integrator were used to quantify the gas. As a control, water equilibrated in air was treated and analysed identically to the samples. The final data were normalized to give an average saturation of one in the meltstream.

The data are reported in Table 1 in terms of the nitrogen and oxygen saturation (S_N and S_O , respectively), and the ratio of the volume concentrations of nitrogen to oxygen. The saturation is defined as the volume concentration of gas divided by the value that would be found in water in equilibrium with moist air at 1 atm total pressure. Unity saturation at 0°C corresponds to $18.42 \text{ cm}^3 \text{ l}^{-1}$ for N_2 and $10.22 \text{ cm}^3 \text{ l}^{-1}$ for O_2 (ref. 9). Both N_2 ($S_N = 1.45$) and O_2 ($S_O = 1.97$) are supersaturated just under the ice cover. At 12 m depth, the saturations increase for both gases to 1.63 for nitrogen and 2.55 for oxygen. The O_2 results are in agreement with those reported by Wharton *et al.*³ for 1978–83.

The reservoir of dissolved gases, both N_2 and O_2 , in the lake water greatly exceeds the annual sources and sinks. As a result, the concentrations of these gases will not change significantly throughout the year, and conditions of supersaturation will prevail even in the winter³. The values at 12 m depth, therefore, represent the long time-period averages for the water in the lake, and can be used to determine that the N_2 turnover time (total concentration divided by yearly input) is $\sim 60 \text{ yr}$. Wharton *et al.*³ have determined that the time constant for turnover of oxygen in the lake water is $\sim 30 \text{ yr}$.

The gas concentrations in the water just below the ice show the effect of mixing of the atmosphere equilibrated meltstream water with the supersaturated lake water. The values of S_N , S_O and the volume ratio just below the ice can be approximated by mixing the meltstream water with lake water in the proportions of 35% and 65%, respectively (Table 1). Mixing is confined to the top few metres in this amictic lake.

We can obtain a rough estimate of the relative strength of the biological and non-biological gas sources by considering the ratio of N_2/O_2 at 12 m to be characteristic of the steady-state value for the entire lake. The steady state concentrations of N_2 and O_2 , determined by balancing the biological and abiological production with the loss rate, can be approximated by

$$k_N[N_2^*](S_N - 1) = A_N + B_N \quad (1)$$

$$k_O[O_2^*](S_O - 1) = A_O + B_O \quad (2)$$

where k is the net loss coefficient, $[N_2^*]$ and $[O_2^*]$ are the equilibrium concentrations by volume of each gas ($[N_2^*]/[O_2^*] = 1.8$), A is the net abiological input, B is the net biological production, and subscripts N and O refer to nitrogen and oxygen, respectively. When the terms on the right-hand side of equations (1) and (2) go to zero, the gas concentrations in the lake go to their equilibrium values. For this approximation, we assume that the loss coefficient for N_2 is the same as that for O_2 . The ratio of the abiological source of nitrogen to the abiological source of oxygen is given by the atmosphere equilibrium ratio ($A_N = 1.8A_O$), and because there is no biological source of nitrogen $B_N = 0$. We can then solve equations (1) and (2), and using the observed values of S_N and S_O , determine that 59% of the net O_2 production in the lake is the result of biological processes ($B_O/(B_O + A_O) = 0.59$) and 41% is the result of abiological processes ($A_O/(B_O + A_O) = 0.41$).

The data reported here can be used to explain the formation of lift-off mat in the lake. Lift-off mat is produced when the pressure of dissolved gases inside the algal mat exceeds the local hydrostatic pressure at that depth and bubbles form inside the mat causing it to tear loose from the lake bottom. The maximum depth, z (in metres), at which bubbles can form is given by³

$$z = 10.3[0.21(S_O - 1) + 0.78(S_N - 1) + p_w] \quad (3)$$

where p_w is the vapour pressure of water in units of atmospheres (negligible). The values for S_N and S_O in Table 1 at 12 m depth give a maximum bubble formation depth of 8.4 m. This is within reasonable agreement with reported maximum depth¹⁰ for lift-off mats of $\sim 11 \text{ m}$. The fact that the maximum reported depth exceeds the calculated value is expected considering the local enhancement of O_2 in the mat by photosynthesis.

To the best of our knowledge, steady-state, large-scale supersaturation of N_2 such as observed in this Antarctic lake has not been previously described. In addition to the perennially ice-covered Antarctic lakes, we predict that certain lakes in the Arctic would be supersaturated with N_2 . These are shallow lakes which have annual ice covers. When the ice cover forms, high oxygen concentrations are observed and have been attributed to dissolution of gases on freezing^{11,12}. As in the Antarctic lakes, N_2 should also be concentrated by this mechanism. Furthermore, as discussed above, the ratio of N_2/O_2 could provide a useful indicator of the relative roles of biological and non-biological processes in determining the gas balance of such lakes.

The Antarctic dry valley lakes have been suggested as analogues for putative ice-covered palaeolakes in the canyon regions of Mars^{13,14}. The floors of the Valles Marineris canyon system contain sedimentary deposits exhibiting rhythmic horizontal layering which suggests deposition in standing water. This hypothesis is consistent with other geological evidence for abundant liquid water early in Mars' history. Because of the similarity between the primordial environments of Mars and Earth it is interesting to speculate on martian environments that may have been suitable sites for the origin of life. McKay *et al.*¹³ have shown that, like the Antarctic lakes, martian lakes would contain liquid water beneath a layer of ice. In addition to providing a relatively warm, liquid water environment, the mechanisms we have described above could have significantly affected the gas budget in those lakes, possibly enhancing the concentrations of biologically important gases from a thin martian atmosphere.

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Developmental stability of insecticide resistant phenotypes in blowfly; a result of canalizing natural selection

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Maynard Smith *et al.*¹ have recently suggested that newly evolved phenotypes will be more variable than long-established ones when developmental constraints have evolved through canalizing selection in which similar phenotypic expression is stabilized across a range of genotypes². There is as yet little evidence for this^{1,3}. Fluctuating asymmetry¹ (random differences between left and right sides of a normally bilaterally symmetric organism) has been used to indicate developmental stability and to define environmental or genetic stresses on development⁴⁻¹⁴. Here we show that insecticide-resistant phenotypes of the Australian sheep blowfly, *Lucilia cuprina*, show greater fluctuating asymmetry than susceptible phenotypes when resistance first evolves, and that continued use of an insecticide after resistance develops leads to a return in the asymmetry of resistant phenotypes to the level of susceptibles. The asymmetry of resistant phenotypes increases during repeated backcrossing to a susceptible strain, suggesting that developmental stability may be modified by selection.

Resistance to dieldrin, a cyclodiene, occurred within two years of its introduction (1955) for blowfly control. The chemical was

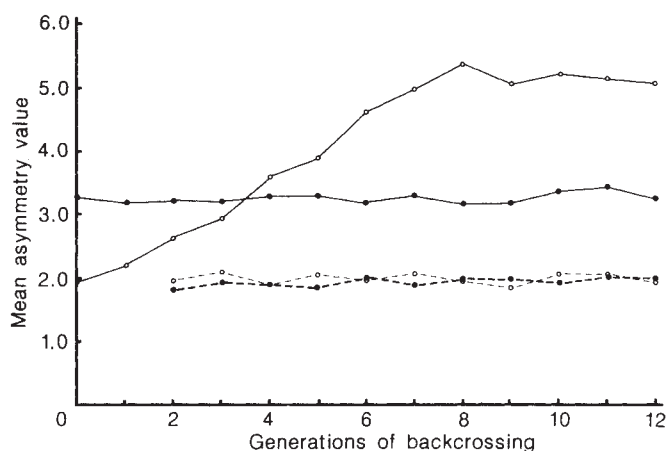


Fig. 1 Asymmetry in resistant heterozygotes and in susceptibles at each generation of backcrossing of *Rop-1/Rop-1* and *Rdl/Rdl* to *M₁5*. Solid lines, resistant heterozygotes: ○, *Rop-1/+*; ●, *Rdl/+*. Broken lines, wild-type blowflies from (○) *Rop-1/Rop-1* and (●) *Rdl/Rdl* backcross. Range of s.e.m. 0.06-0.10.

replaced by diazinon, an organophosphate, which has been used since in spite of resistance developing in 1967 (ref. 15). Resistance in the sheep blowfly to these two insecticides is largely determined by alleles of two unlinked genes (diazinon, *Rop-1*, chromosome IV; dieldrin, *Rdl*, chromosome V)^{16,17}.

The strains used in this study were SWT, a susceptible strain genotypically *+/+* at each resistance locus; *M₁5*, with the same susceptibility genotype as SWT but homozygous for a recessive marker on each autosome (II black puparium (*bp*); III rusty body (*ru*); IV golden body (*gl*); V *M₁* veinless (*m₁*); VI yellow eyes (*y*)¹⁸); and pure breeding diazinon (*Rop-1/Rop-1*) and dieldrin (*Rdl/Rdl*), resistant strains collected in north central Victoria in 1981. Fluctuating asymmetry was estimated from the absolute difference in bristle count between the left and right sides of the frontal head stripe, the outer wing margin and the *R₄₊₅* wing vein. There was no evidence of directional asymmetry and similar values of asymmetry, independent of character mean, are found in each sex for each character. There was no significant difference in asymmetry between SWT, *M₁5* and *Rop-1/Rop-1*. However each was significantly less asymmetric than *Rdl/Rdl* (Table 1). These values have been stable across 40 generations.

To test the effects of genetic background on asymmetry *Rop-1/Rop-1* and *Rdl/Rdl* females were crossed to *M₁5* males. Progeny of each cross were treated with insecticide to discriminate *+/+* from *R/+*^{16,19} and asymmetry scored for 50 *+/+* and 50 *R/+* of each sex where *R* represents a resistance allele. Heterozygous females were then backcrossed to *M₁5* males. The procedure was repeated for 12 generations, with three replicates for *Rop-1/Rop-1* and two for *Rdl/Rdl*. Mean asymmetry of *Rdl/+* and *+/+* stayed constant at the levels of the parental strains (*Rdl/Rdl* and *M₁5* respectively) indicating that the disruptive effect of the *Rdl* allele on development is dominant and not significantly influenced by genetic background. Mean asymmetry of *Rop-1/+* increased and reached a plateau, at a value

Table 1 Mean asymmetry insecticide-resistant and susceptible strains of blowfly

SWT	1.83 ± 0.08
<i>M₁5</i>	1.81 ± 0.07
<i>Rop-1/Rop-1</i>	1.92 ± 0.07
<i>Rdl/Rdl</i>	3.23 ± 0.08

Calculations are the absolute difference between left and right side scores pooled over 3 bristle characters. Values are shown as the mean of data for 150 flies ± s.e.m.

Table 2 Asymmetry of chromosomal classes in progeny of a test cross of *Rdl/Rdl* or *Rop-1/Rop-1* with M_1S

	<i>Rdl/Rdl</i> × <i>M</i> ₁ <i>S</i>			<i>Rop-1/Rop-1</i> × <i>M</i> ₁ <i>S</i>	
	<i>+/+</i> (<i>m</i> ₁ / <i>m</i> ₁)	<i>Rdl/+</i> (<i>m</i> ₁ ⁺ / <i>m</i> ₁)		<i>+/+</i> (<i>gl/gl</i>)	<i>Rop-1/+</i> (<i>gl</i> ⁺ / <i>gl</i>)
<i>bp/bp</i>	1.84 ± 0.007	3.23 ± 0.008	<i>bp/bp</i>	1.87 ± 0.004	2.54 ± 0.122
<i>bp/bp</i> ⁺	1.83 ± 0.007	3.25 ± 0.006	<i>bp/bp</i> ⁺	1.87 ± 0.006	2.56 ± 0.116
<i>ru/ru</i>	1.85 ± 0.007	3.24 ± 0.005	<i>ru/ru</i>	1.87 ± 0.004	3.21 ± 0.004
<i>ru/ru</i> ⁺	1.82 ± 0.005	3.24 ± 0.008	<i>ru/ru</i> ⁺	1.87 ± 0.006	1.89 ± 0.011
<i>gl/gl</i>	1.84 ± 0.006	3.25 ± 0.005	<i>m</i> ₁ / <i>m</i> ₁	1.86 ± 0.005	2.55 ± 0.089
<i>gl/gl</i> ⁺	1.83 ± 0.007	3.23 ± 0.008	<i>m</i> ₁ / <i>m</i> ₁ ⁺	1.88 ± 0.005	2.55 ± 0.120
<i>y/y</i>	1.84 ± 0.007	3.25 ± 0.005	<i>y/y</i>	1.87 ± 0.005	2.54 ± 0.120
<i>y/y</i> ⁺	1.84 ± 0.007	3.23 ± 0.008	<i>y/y</i> ⁺	1.87 ± 0.005	2.56 ± 0.117

Values are means ± s.e.m.

greater than that for dieldrin resistance, after 7 generations of backcrossing (Fig. 1). The asymmetry of +/+ at this locus stayed constant at the level of the M_1S strain. After 7 generations of backcrossing less than 1% of the field genome is intact. The *Rop-1* allele may therefore have had a deleterious influence on development when it first arose but this has since been modified by selection. As in the case for the *Rdl* locus, the initial effect of the *Rop-1* allele on asymmetry is dominant. *Rop-1/Rop-1* genotypes constructed after 12 generations of backcrossing have an asymmetry value of 5.09 ± 0.08 , a value identical to that of *Rop-1/+* at this generation (Fig. 1).

In separate experiments, each involving two replicates, *Rop-1/Rop-1* and *Rdl/Rdl* females were crossed to M_1S . F_1 males were testcrossed with M_1S and the mean asymmetry of 20 progeny of each sex scored for each of the 32 phenotypic classes defined by the markers described previously. Asymmetry in progeny of the *Rdl/Rdl* cross follows the segregation of the *Rdl* allele. *Rdl/+* genotypes, m_1^+/m_1 with respect to the chromosome V marker, are significantly more asymmetric than +/+ genotypes for each comparison within the other chromosome classes (Table 2). Factorial analysis shows the m_1^+ chromosome explains over 99% of the variation in asymmetry and that modifiers are hence of little importance. There is homogeneity within chromosome classes for the +/+ genotypes of the *Rop-1/Rop-1* cross. However, the chromosomes vary in their effect on the *Rop-1/+* genotype (Table 2). The segregation of chromosome III influences asymmetry; *ru/ru* has significantly higher asymmetry than the other chromosome classes while *ru/ru*⁺ has significantly less. The intermediate asymmetry and higher standard error of the other classes reflect bimodal distributions of chromosome III segregation (for example, *bp/bp ru/ru gl/gl*⁺, 3.21 ± 0.006 ; *bp/bp ru/ru*⁺ *gl/gl*⁺, 1.86 ± 0.007). The difference in asymmetry between the genetic localization (Table 2) and the backcross experiments (Fig. 1) may indicate the presence of modifiers linked to *Rop-1* and possible epistatic interactions. However, the mean asymmetry in the diazinon crosses is strongly influenced by the segregation of the *Rop-1* allele and of chromosome III.

Before insecticide use, resistant genotypes are at a disadvantage and resistant alleles are very rare in populations²⁰⁻²². Newly arisen resistance alleles influence biochemical and physiological processes²⁰ and may hence disrupt development. After resistance becomes common, continued selection by the insecticide may favour modifications minimizing the deleterious effects of the new allele and resulting in increased relative fitness of resistant genotypes even in the absence of insecticide²¹. In *L. cuprina* such modifiers of fitness are on chromosome III (ref. 22) as is the modifier(s) of asymmetry (Table 2). For dieldrin resistance, there is no indication of such modification of fitness (J.A.McK. and A. Purvis, unpublished data) or asymmetry (Table 2) presumably because the chemical was used only for a limited period after resistance developed²³. Modifications of diazinon resistant genotypes may reflect the long period of use after resistance appeared. Similar patterns of pesticide use have occurred in other systems²¹ and could be used to test the generality of our

results and provide further evidence of the importance of modifiers in the evolutionary process²⁴.

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Symbiosis of methylophilic bacteria and deep-sea mussels

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Recently, dense assemblages of seep mussels and other benthic invertebrates resembling hydrothermal vent communities were found associated with reducing sediments at hypersaline seeps in the abyssal gulf of Mexico¹. A symbiotic association with sulphur-oxidizing chemolithoautotrophic bacteria, similar to those recently reported (for review see ref. 2), was postulated for the seep mussel. However, the very negative $\delta^{13}C$ values reported for the mussels (-74%) (ref. 3) suggested that these symbioses were different from those reported for bivalves from hydrothermal vents and reducing sediments (where $\delta^{13}C$ ranges from -23 to -34% ; refs 4, 5). Here, we present microscopical and enzymatic evidence supporting the hypothesis that methylophilic bacteria capable of using reduced C-1 compounds as their carbon and energy sources, occur as intracellular symbionts of the seep mussel.

The Gulf of Mexico mussels appear to belong to a new species of mytilid different from the hydrothermal vent species (R. D. Turner, personal communication). The abnormally low $\delta^{13}C$ values³ for the mussel tissue has fuelled speculation that biogenically produced methane ($\delta^{13}C$ of -60 to -90%)⁶ might be the source of this depleted carbon. As no animals are known that can utilize methane as a carbon source, a symbiosis was proposed to exist between the mussels and methane-utilizing bac-

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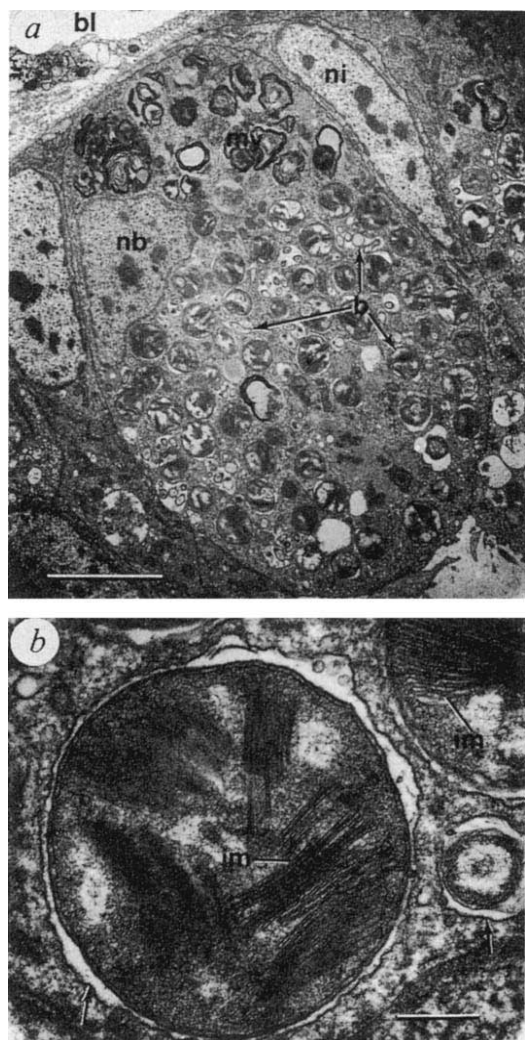


Fig. 1 Transmission electron micrographs showing bacteria-like bodies (presumed symbionts) within gill epithelial cells of the Florida Escarpment mussel. *a*, Transverse section, slightly oblique of gill filament showing host cells containing symbionts (bacteriocytes). Bacteriocytes are typically separated by cells lacking symbionts (intercalary cells) and occasional mucous cells (not shown). Two size classes of symbionts are observed (arrows): large coccoid-shaped cells which contain complex intracellular membranes; and small coccoid- or rod-shaped bacteria. The densities of the larger symbionts, as judged by nucleic acid staining is $\sim 3 \times 10^8$ per g gill fixed weight. Myelin-like structures are typically observed in the basal region of the bacteriocytes. Infrequently, rod-shaped bacteria $\sim 0.4 \mu\text{m}$ in diameter and $\leq 8 \mu\text{m}$ in length occurred densely packed within a basal membrane-bound vacuole (not shown). Scale bar, $5 \mu\text{m}$. *b*, Bacteria-like symbionts; *bl*, blood space; *my*, myelin-like figures; *nb*, nucleus of bacteriocyte; *ni*, nucleus of intercalary cell. *b*, Higher magnification, transverse sections of both large and small symbionts, showing cell ultrastructure typical of Gram-negative bacteria. The symbionts are contained within vacuoles, either singly or in groups of two or more, surrounded by a peribacterial membrane (arrows) presumed to be derived from the host. Note stacks of complex intracytoplasmic membranes in large symbionts. Scale bar, $0.3 \mu\text{m}$; *im*, intracytoplasmic membranes.

Methods. Intact mussels were collected from the base of the Florida Escarpment with DSRV Alvin (dive 1343, 9 Mar 1984, 3,266 m depth). Gills were dissected on board ship by J. Hook, fixed and stored for 2.5 weeks in 3% glutaraldehyde in 0.1 M cacodylate buffer with 0.4 M NaCl, then postfixed in OsO_4 , embedded in Spurr's resin, and sections stained with lead citrate and uranyl acetate.

teria, similar to the symbioses discovered between mussels and sulphide-utilizing bacteria². We examined Florida Escarpment mussel tissue for symbionts with ultrastructure typical of methane-oxidizing bacteria.

The gills of these mussels are similar to symbiont-containing gills of other vent and nearshore bivalves^{2,7} in that they are large, thick and fleshy. Transmission electron microscopic (TEM) examination of this tissue reveals the presence of numerous bacteria-like bodies (Fig. 1), subcellular inclusions having the same general size and ultrastructure as bacterial cells, including a cell envelope typical of Gram-negative bacteria. These inclusions (here in after symbionts) are variable in size and ultrastructure (Fig. 1) but generally fall into two classes: (1) large coccoid-shaped cells, about $1.6 \mu\text{m}$ in diameter, containing bundles or stacks of complex intracytoplasmic membranes, and (2) small coccoid- or rod-shaped cells, about $0.4 \mu\text{m}$ in diameter, without internal membranes. Symbiont division stages were observed but only rarely. Gill smears and homogenates, stained with the nucleic-acid-specific stain 4',6-diamidino-2-phenylindole (DAPI)⁸ revealed uniformly fluorescent spherical cells with the same dimensions as the large symbionts; smaller fluorescent particles were also observed in these preparations, but these could not be distinguished from mitochondria. The large fluorescent cells were not observed in tissue homogenates of foot or mantle tissues indicating that, as in chemolithoautotroph-bivalve symbioses^{2,7}, the symbionts appear restricted to the gills.

The general ultrastructural organization of the symbionts in the gill tissue is similar to that described for other bivalves^{2,9-11} and is described only briefly here. All the symbionts appeared intracellular, in specialized gill epithelial cells (bacteriocytes), in vacuoles surrounded by a peribacterial membrane either singly or in groups of two or more (Fig. 1). The bacteriocytes occur in specific regions of the gill filaments, alternating with symbiont-free cells (intercalary cells and/or mucous cells). Apparent degradation stages of symbionts occurred regularly, particularly in the basal region of the bacteriocytes where myelin-like figures commonly occurred (Fig. 1*a*). The intracytoplasmic membranes seen in the large symbionts are reminiscent of those seen in several bacteria, including type I methane-oxidizing bacteria^{12,13}.

We tested mussel tissues for the presence of key enzymes from methylotrophic and autotrophic pathways. The mussels were collected (dive 1346, 12 March 1984) and frozen on board ship at -20°C , transferred to the laboratory and stored at -80°C . Assays were conducted 1–1.5 yr after collection. Specific tissues dissected from frozen mussels were homogenized by hand in assay buffer on ice and the homogenates were either used directly for assays or in the preparation of cell-free extracts. The supernatant that remained after homogenates were sonicated ($4 \times 30\text{s}$) or run through a French Press ($2 \times 20,000$ p.s.i.) and centrifuged for 5–15 min at 12,000–15,000g was used for assays. Protein was determined by the method of Lowry¹⁴ using bovine serum albumin as a standard.

To convert methane to multicarbon compounds, enzyme activities are necessary for oxidizing and assimilating C-1 compounds¹⁵. We were unable to detect methane oxidation by mussel tissue homogenized in 20 mM Tris HCl buffer, pH 8.0, either using $^{14}\text{CH}_4$ (ref. 15) or a cell-free methane mono-oxygenase assay¹⁶. This is not surprising as the tissues had been frozen for more than 1 yr, and methane oxidation activity is quite labile in free-living methane-oxidizers¹⁷. However, methanol dehydrogenase activity was detected in five gill samples from two different mussels ($4\text{--}32$ nmol per min per mg protein; detection limit = 0.1 nmol per min per mg protein) but not in two mantle tissue samples or one foot tissue sample. This activity resembled the classical pyrroloquinoline quinone-linked methanol dehydrogenase found in a wide range of methylotrophic bacteria as it was ammonia-dependent, had a pH optimum of 9.0 and showed activity with formaldehyde and ethanol^{13,18}. NAD-

linked formate dehydrogenase activity¹⁸ and NAD- and glutathione-linked formaldehyde dehydrogenase activities¹⁸ were detected in some samples but were variable.

Two major carbon assimilatory pathways exist in methane-oxidizers: the ribulose monophosphate pathway, found in type I strains; and the serine cycle, found in type II strains. The key enzyme for the first pathway, hexulose phosphate synthase, was detected at high levels in gill tissue (132–314 nmol per min per mg protein), in five samples from three mussels, using a coupled assay¹⁹, but was not detected in mantle or foot tissue. Enzymes of the serine cycle, hydroxypyruvate reductase²⁰, and serine-glyoxylate aminotransferase²¹ were either not detectable or were present at variable concentrations, but were never found together. The key enzyme of autotrophic assimilation, ribulose biphosphate carboxylase, was not detectable in any sample, using two different assay methods^{22,23} under a variety of assay conditions and methods of lysis.

The presence of enzyme activities unique to assimilatory and dissimilatory pathways of methylotrophic metabolism and the absence of detectable ribulose biphosphate carboxylase strongly suggest that methylotrophic metabolism occurs in the gills of the seep mussels. The co-occurrence of type I methylotrophic enzyme activities, type I intracytoplasmic membrane-containing symbionts and low $\delta^{13}\text{C}$ values in mussel tissue also suggest that methane-oxidizers exist in symbiosis with these mussels, and probably provide a major source of nutrition. The identity of the other bacteria-like structures seen in the electron micrographs remains to be determined.

Although the distribution and occurrence of methylotrophic symbioses is not known, similar types of membranes have been observed in bacterial symbionts of one pogonophore²⁴, and low $\delta^{13}\text{C}$ values have been noted for these and other marine invertebrates^{3,5,25}. Although further characterization is necessary to determine the nature of these associations, the benefits afforded both partners suggest that symbioses between invertebrates and methylotrophs may be widespread in nature.

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Note added in proof: Recently another species of symbiont-containing mussel from the Louisiana slope of the Gulf of Mexico has been shown to consume methane²⁶.

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Peptides containing the cell-attachment recognition signal Arg-Gly-Asp prevent gastrulation in *Drosophila* embryos

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It has recently been suggested that the Arg-Gly-Asp sequence (RGD) forms part of a widespread cell–extracellular matrix recognition system (see for example ref. 1). Analysis of the cell binding sites of vertebrate fibronectin^{2,3} and other extracellular proteins that interact with cell surfaces implicate the same amino acid triplet¹. Peptides containing this sequence inhibit certain developmental events such as cell–matrix adhesion or cellular migration *in vitro*^{2–7} and *in vivo*⁸. The RGD-sequence is also part of the cellular recognition site of the aggregation protein discoidin I in *Dictyostelium*⁹ suggesting that the RGD-recognition system could be universally used. In *Drosophila*, despite its advanced genetics, very little is known about the extracellular components that are involved in cell movements and morphogenesis. We report here that peptides containing the RGD-sequence prevent gastrulation of *Drosophila* embryos. The phenotypic effect is similar to that observed in the dorsal-group mutants: no ventral furrow is formed and the embryos lack dorsal–ventral polarity. The specificity of the inhibiting action suggests that the RGD-sequence may also be used by invertebrates to mediate cell-attachment phenomena.

Early *Drosophila* embryogenesis is initiated by 13 rapid and synchronous nuclear cleavages which occur within a syncytium. At the end of the 13th cycle, cellular membranes begin to form at the periphery of the embryo; their completion defines the cellular blastoderm¹⁰. Soon after, gastrulation starts with the invagination of mid-ventral blastoderm cells. An integrated series of complex movements follows, consisting of 4 other invaginations which produce the major rudiments of the endoderm¹¹. Cellular adhesion is presumably of crucial importance in these different morphogenetic movements characterizing gastrulation.

Injections of synthetic RGD-peptides were therefore carried out at the syncytial blastoderm stage. RGD-containing peptides injected in the ventral periplasm of the embryos prevent gastrulation (Table 1). Our criterion for gastrulation was the appearance of the ventral furrow, which is readily observed by light microscopy. As embryos without a ventral furrow never hatch, we designate these defective embryos as non-viable or non-gastrulating. Control peptides lacking the RGD-sequence have no effect on the viability of embryos, even at concentrations 50 times higher than those effective with RGD-peptides, and are not more harmful than the injection medium alone. Also, Arg-Gly-Asp-Ser (RGDS) is as efficient as longer peptides suggesting that, as in cultured vertebrate cells^{2,3}, the active sequence contains the RGD-sequence. The specificity of this sequence is best demonstrated by the peptide Gly-Arg-Gly-Glu-Ser, where the

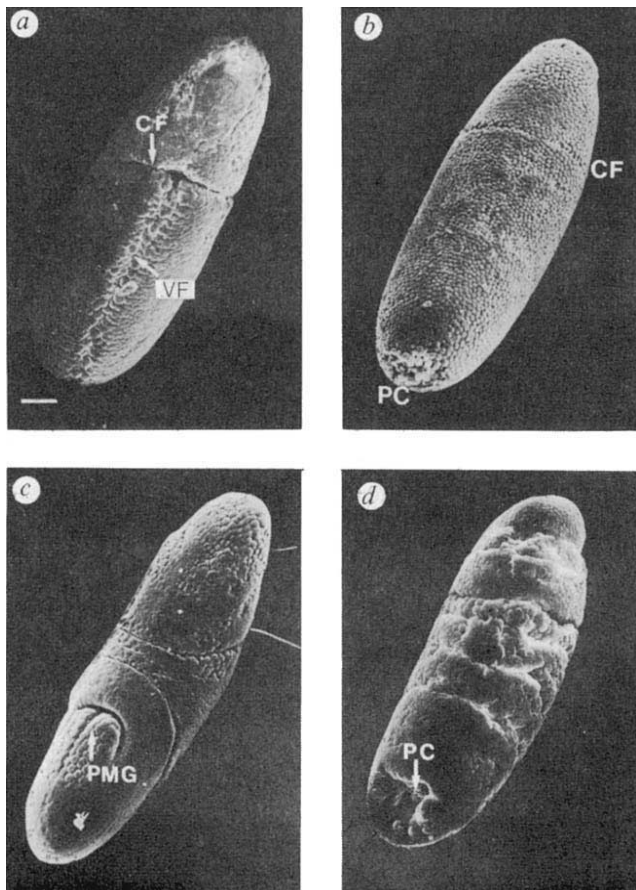


Fig. 1 Scanning electron microscopy of normal and arrested *Drosophila* embryos. *a*, Ventral view of a 3.5-h embryo injected with a control peptide showing the ventral furrow (VF) and the cephalic furrow (CF) which are both already visible at 3 h. *b*, Ventral view of an embryo injected with RGD-containing peptide. No ventral furrow is formed in an embryo of the same age as in *a*, but a fold (CF) which might be the cephalic furrow begins to appear at that time. *c*, Dorsal view of a 4.25-h embryo injected with a control peptide. The post-midgut rudiment (PMG) is moved anteriorly by the elongating germ band. *d*, Dorsal view of a 4.25-h embryo injected with RGD-containing peptide. This embryo is symmetrical in the dorsal-ventral axis. Its orientation can only be ascertained by the curvature which normally occurs on the ventral side of the embryo. None of the multiple folds visible at the surface of the embryo seem to correspond to normal invaginations seen in *c*. The distribution of the invaginations does not follow a well-established pattern. Polar cells (PC) are internalized at the posterior tip of the embryo instead of being carried along the dorsal surface by the migration of the posterior midgut rudiment. This abnormal location of the polar cells seems to be a constant feature of RGD-embryos. For scanning electron microscopy, the embryos were prepared according to Turner and Mahowald²³, after careful removal by toluene of the mineral oil used for microinjection. Following critical point drying, the embryos were coated with gold and examined with a JEOL JSM 35 scanning electron microscope at 15 kV; scale bar, 20 μ m.

replacement of Asp by Glu is sufficient to abolish the inhibiting effect on gastrulation.

The peptides affecting gastrulation do not appear to be cytotoxic, as the incorporation of ³H-thymidine or ³H-uridine in treated embryos strictly follows the same kinetics as for untreated embryos, at least during the first six hours of development (data not shown).

The inhibiting concentrations of peptides correspond to doses even lower than those that block gastrulation in *Pleurodeles*⁸. If injected stock solutions are assumed to be uniformly diluted in the embryo, the concentration for optimal inhibition by Gly-Arg-

Gly-Asp-Ser is 10 μ M. As peptides probably do not diffuse freely inside the embryo, the effective concentration would probably be similar to those for cultured vertebrate cells ($\sim$ 50 μ M for half-maximal inhibition of the spreading of BHK cells³ and $\sim$ 250 μ M for NRK cells²).

The representative phenotype of embryos injected with RGD-containing peptides (RGD-embryos) is shown in Fig. 1. An apparently normal cellular blastoderm is formed at the proper time (2.75 h) in RGD-embryos. Embryos injected with a control peptide (C-embryos) start forming ventral and cephalic furrows within 5–10 min after the cellular blastoderm. As shown in Fig. 1*a*, these invaginations are completed 3.5 h after fertilization, and control embryos have initiated germ band extension on the dorsal side. For RGD-embryos, only one invagination becomes visible at that time in the anterior part (Fig. 1*b*) and may correspond to the cephalic furrow, even though it seems somewhat different from that normally observed. During this period, the embryos resemble a normal blastoderm that persists for an abnormally long time (45 min $\pm$ 15 min). About one hour later, instead of the characteristic invaginations of a control embryo (Fig. 1*c*), multiple disorganized folds can be observed in the ectoderm all around the circumference of the embryo (dorsal view in Fig. 1*d*). The polar cells do not migrate dorsally following the posterior midgut rudiment but instead are internalized directly at the posterior tip of the embryo (Fig. 1*d*).

Phenotypically, RGD-embryos behave as if they were symmetrical along the dorsal-ventral axis. Later in development, no muscular movements are observed and no internal organs are formed. These late effects are most probably a consequence of the lack of invagination of the mid-ventral blastoderm cells, which prevents the formation of mesoderm. This interpretation is supported by examination of semi-thin sections of 3.5-h RGD-embryos (data not shown). We have not exhaustively searched for intermediate phenotypes. However, when lower doses of the peptide Gly-Arg-Gly-Asp-Ser were injected in the ventral periplasm, only the proportion of viable embryos was increased. Defective embryos still had no ventral furrow, rather than an incomplete one.

The identity and the nature of the molecule(s) bearing the RGD-sequence in *Drosophila* remain to be established. By analogy with other well known systems, it is tempting to postulate a fibronectin-like role for the RGD-bearing molecule(s) in cell adhesion during the gastrulation step. Some basement membrane components have been identified in *Drosophila* embryos¹², but none of them is present at gastrulation and no fibronectin-like protein(s) has been identified. Our experiments to characterize such proteins have failed. This suggests that even though fibronectin has been well conserved during evolution, insect proteins may have preserved only the RGD-cell recognition signal sequence. Experiments are now in progress to isolate the RGD-bearing molecule(s).

The forces involved in the movements of gastrulation are not yet known, but several hypotheses have been proposed invoking some contractile system, change of cell shape¹¹ and direct involvement of cytoplasmic yolk bridges¹³. The inhibiting effect of RGD-peptides described here suggests that cell adhesion mediated by interaction with an extracellular matrix could in addition be implicated, at least as far as ventral furrow formation is concerned.

Finally, the phenotype of RGD-embryos was found to resemble closely that of the dorsal-group mutants. Ten loci have been identified for maternal effect mutations which fail to establish normal dorsal-ventral polarity^{14,15}. As for at least two other zygotic mutants, they do not form the ventral furrow¹⁶ and have consequently also been termed gastrulation defective¹⁵. Figure 2 shows scanning electron micrographs of one dorsal-group mutant, *snake*, at stages comparable to those depicted in Fig. 1. Strong similarities can be observed: no ventral furrow is formed; the appearance of the cephalic furrow is delayed compared with the wild-type embryo; multiple folds in the ectoderm

Table 1 Microinjection of synthetic peptides into early *Drosophila* embryos

	Initial peptide concentration (mg ml ⁻¹)	Injected embryos	Gastrulating embryos	Gastrulating embryos (%)
<u>Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Lys-Pro</u> (P1)	1	378	53	14
Cys-Glu-Asp-Ser-Glu-Thr-Arg-Thr-Phe-Tyr (P3)	10	372	328	90.6
Gly-Arg-Gly-Asp-Ser	0.2	72	9	12.5
Gly-Arg-Gly-Glu-Ser	0.2	85	78	91.70
<u>Arg-Gly-Asp-Ser</u>	1	51	4	7.8
Tyr-Ala-Gly-Gly	10	39	34	87.2
Injection medium (0.2 nl)	—	210	191	91

Staged embryos were obtained from Oregon R flies at 25 °C, manually dechorionated, placed on a microscope slide coated with Scotch-tape glue and desiccated for 5 min in a CaCl₂ atmosphere. They were covered with mineral oil at 18 °C and observed under an inverted light microscope. Embryos were microinjected with the aid of a De Fonbrune micromanipulator with a 1 µm glass needle. An average volume of 0.2 nl, corresponding to 2.5% of the total volume of the egg, was delivered to each embryo. Only one concentration of each peptide was used, except for the peptide Gly-Arg-Gly-Asp-Ser which has been also injected at lower concentrations (see text). P1 is a specific and highly conserved amino-acid sequence of vertebrate fibronectin which contains the cell-attachment recognition site³. P3 is another highly conserved sequence in the collagen-binding domain of fibronectin and is not related to the attachment of fibronectin to the cells. The injection medium was 17 mM NaCl, 10 mM KCl, 1 mM MgCl₂ and 2.5 mM CaCl₂ at pH 7.2. Injections were carried out in the ventral periplasm of embryos at stage 13 according to Zalokar and Erk¹⁰ during the 12th cleavage cycle which lasts 13 min (ref. 22). Ventral furrow formation was monitored by light microscopy for 2 h after the onset of the cellular blastoderm. Underlined, RGD sequences.

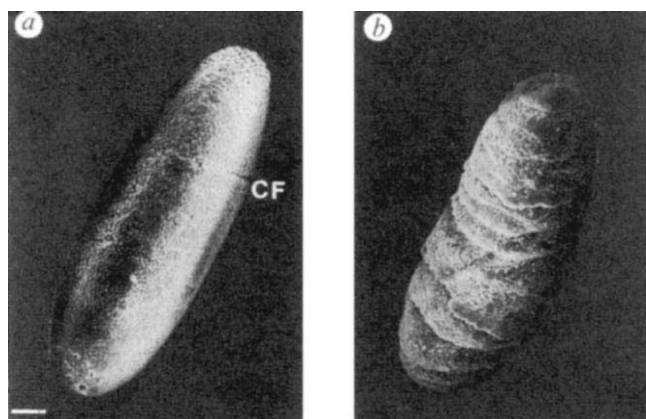


Fig. 2 Scanning electron microscopy of *snake* mutant. Experimental conditions as in Fig. 1. *Snake* embryos were collected from homozygous females carrying the strong allele *snk*⁰⁷³ (gift of Dr Nüsslein-Volhard). *a*, Ventral view of a 3.5-h *snake* embryo showing no ventral furrow. Cephalic furrow (CF) begins to appear at that time. *b*, Dorsal view of a 4.5-h *snake* embryo. As for the embryo shown in Fig. 1d, the folds extend around the whole circumference of the embryo. The orientation of the embryo in this photograph does not show the internalization of the polar cells, which occurs in a similar way as for RGD-embryos.

are produced and the polar cells stay at the posterior tip of the embryo. The analogy can be pursued further with cuticle preparations, which show the same general features as for the most severe alleles of the dorsal-group. RGD-embryos can secrete cuticle but only structures corresponding to dorsal cuticle can be distinguished (data not shown). As cuticle deposition is a late event that arises well after gastrulation, this suggests that at least some cells in the embryo have undergone an adequate differentiation programme. The absence of ventral cuticle in the dorsal-type mutant has been shown to result from an alteration of the final differentiation fate of blastoderm cells¹⁷. Assuming that the same is true for RGD-treated embryos, our data suggest that the developmental programme of the ventral blastoderm cells can also be modified by affecting the cell-matrix interactions responsible for normal gastrulation.

Genetic analysis supports a picture of *Drosophila* gastrulation as a cascade of events: affecting any single step leads to the same ultimate morphological defects. Is one of the dorsal gene products directly involved in cell-matrix interaction? Direct involvement of a purely maternal gene product seems very unlikely. RGD-peptides are effective not only at gastrulation but also at other times in *Drosophila* embryonic development (C.N. and M.S., in preparation), suggesting instead the participation of a zygotic gene product. Molecules involved in cell-matrix recognition are often ubiquitous components that intervene in various morphogenetic processes¹⁸ and at different times in development¹⁹. Thus, if specific gastrulation gene products are related to a cell-matrix recognition system, they are more likely to be regulators rather than actual components of this recognition system. Rapid progress in the molecular genetics of

the dorsal genes^{20,21}, together with an identification of the RGD-bearing molecules, will probably help to understand better the control of the morphogenetic processes of gastrulation.

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Spontaneous recovery after experimental manipulation of the plane of beat in sperm flagella

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It is generally accepted that the oscillatory beating characteristic of sperm flagella is the result of an ATP-induced sliding between the doublet microtubules of the flagellar axoneme^{1,2}, with these longitudinal forces being converted into a lateral bending moment by resistive components of the structure that limit the displacement. However, little is known about the mechanisms that regulate this sliding among the nine doublets of the cylindrical axoneme³ to produce the coordinated planar bending waves required for efficient sperm propulsion. We have investigated these mechanisms with a new procedure in which the sperm head is held in the tip of a vibrating micropipette. Data obtained by gradually rotating the plane of imposed vibration around the sperm axis indicate that the pattern of active sliding between the outer doublet tubules can rotate relative to the sperm head, and suggest that this active sliding is regulated in part by the central tubule complex.

In our experimental arrangement the tip of the sperm head is held in the tip of a micropipette by gentle suction, with the planar flagellar beat⁴ lying approximately within the focal plane of the microscope. The micropipette is oscillated by a piezo-electric driver capable of vibrating along any axis at frequencies up to 150 Hz, with the direction being determined by the distribution of sinusoidal voltage applied to the three sets of piezo elements⁵. We describe here the effects of changing, either abruptly or gradually, the direction of pipette vibration from lateral (within the natural flagellar beat plane) to vertical (perpendicular to the natural beat plane). The vibration frequency was kept approximately equal to the natural beat frequency.

When lateral vibration of the pipette was initiated, the imposed movement of the head was within the plane of beat and the flagellum continued to beat almost normally. Switching the direction of pipette vibration abruptly from lateral to vertical caused a corresponding change in the plane of flagellar beating so that within 50 ms (~ 2 beat cycles) it almost coincided with the new plane of imposed vibration (Fig. 1). When the vibration direction was reset to lateral, the flagellar beat plane returned to the plane of focus equally quickly. This change in orientation of the waveform appears to be an active phenomenon dependent upon flagellar beating, as it occurred both with live sperm swimming in sea water and with demembrated sperm reactivated with exogenous ATP⁶, whereas sperm with their flagella bent into stationary rigor waves⁷ showed no change in the orientation of their waveform under the same conditions.

To investigate this unexpected plasticity of the flagellar beat plane, we varied the amplitudes and phases of the voltages applied to the lateral and vertical piezo elements in a coordinated manner so that the plane of pipette vibration was initially parallel to the natural beat plane of the flagellum and then rotated gradually and continuously in a clockwise or anti-clockwise direction for several revolutions. The beat plane of the flagellum responded by rotating along with the vibration plane of the pipette, following it for at least four complete revolutions (Fig. 2). The direction of flagellar asymmetry, as indicated by the bends on one side of the flagellum being of greater angle than those on the other side^{6,8}, rotated along with the plane of beat. No other change in the parameters of beating was detected. When the vibration was stopped after the flagellum had been

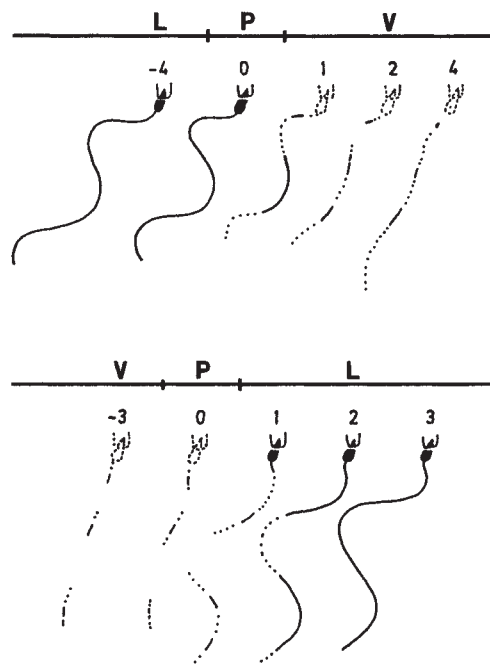


Fig. 1 Tracings of videotaped flagellar images showing the effect of abruptly changing the direction of induced vibration from lateral (L) to vertical (V) and back. There was a brief stationary period (P) of ~ 20 ms between the two directions of vibration. Regions of the flagellum that were partially out of focus are indicated by broken lines. Numbers, fields on the videotape. The live spermatozoon of the sea urchin *Hemicentrotus pulcherrimus* has the tip of its head held by suction in the tip of a micropipette as indicated. It was observed in Ca^{2+} -free artificial sea water using a $\times 40$ phase-contrast objective with illumination by a stroboscopic flash lamp. Frequency and amplitude of pipette vibration, 43.4 Hz and $\pm 4.7 \mu\text{m}$, respectively; mean distance of pipette from cover glass, $13 \mu\text{m}$; flagellar beat amplitude, $\pm 6.7 \mu\text{m}$; flash frequency 42.7 Hz.

'wound up' in this way, the plane of beat spontaneously unwound back to its original orientation. The first turns were reversed within ~ 2 s (Fig. 3), but the final 1–2 turns often unwound more slowly with the interspersing of one or more hesitations at orientations of metastable equilibrium before attaining the natural beat plane. It appeared to make no difference whether the initial direction of rotation was clockwise or anti-clockwise. In some sperm, used as controls, the plane of flagellar beat was first rotated clockwise for several revolutions and then, without stopping, rotated back anti-clockwise for the same number of revolutions; in these cases, the plane of beat remained stable with no spontaneous rotation after the vibration was turned off.

These data indicate that the hydrodynamic forces on the beating flagellum make it energetically favorable to shift its plane of beat to coincide with the plane of motion imposed on the sperm head by the vibrating micropipette, and that this is effected with no slippage between the head and the micropipette. In accomplishing this manoeuvre, the flagellum encounters an elastic restraining force of magnitude insufficient to prevent several complete revolutions of the beat plane, but sufficient to restore the original beat plane when the forcing vibration is terminated. Possible mechanisms for the rotation of the beat plane include twisting of the entire $9+2$ axonemal structure in its basal region, or rotation of the pattern of active sliding among the outer doublet tubules of the axoneme without any twisting of the cylinder of doublet tubules itself. It seems improbable that the first mechanism could allow as much as four complete revolutions of the beat plane without irreversible damage to the

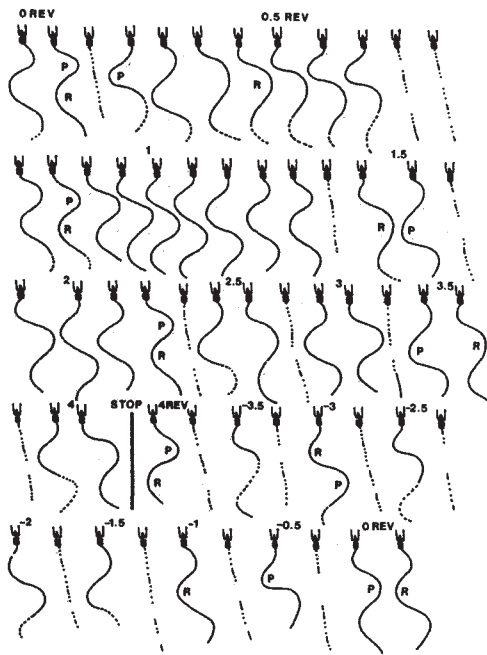


Fig. 2 Selected tracings of a live spermatozoon showing four revolutions of its flagellar beat plane induced by gradual clockwise rotation of the direction of pipette vibration, followed by cessation of the vibration (STOP) and the subsequent spontaneous unwinding of the beat plane. P and R, principal and reverse bends⁶, respectively. It took 204 s to complete the winding process by manually controlling the lateral and vertical components of the driving voltage. The first 3.5 turns of unwinding were complete within 6 s, and the final 0.5 turn occurred 10 s later. Mean distance of the pipette from the cover glass, 33 μm .

motile apparatus or at least a substantial change in the parameters of beating. Thus, we think it more likely that the observed rotation of the beat plane results primarily from a rotation in the pattern of sliding among the doublets, although twisting of the entire axonemal structure may make an additional minor contribution.

Although there is as yet little direct evidence regarding the mechanisms regulating tubule sliding to produce particular patterns of coordinated bending³, the orientation of the plane of beat in flagella and cilia of several organisms is known to be correlated with and perpendicular to the plane of the central pair of tubules⁹. On the basis of electron microscopy of fixed samples, Omoto and Kung have proposed a model in which the sliding of outer doublet tubules associated with the three-dimensional beat of *Paramecium* cilia is regulated by a continuous rotation of the central pair¹⁰, and Kamiya has observed rotation of the central pair during its extrusion from flexing flagella of *Chlamydomonas*¹¹. On the other hand, Tamm and Tamm¹² have shown that no rotation of the central pair occurs during the change from normal to reverse beating in ctenophore cilia.

We suggest that in sea urchin sperm the central pair do not normally rotate but that the forced rotation of the beat plane in our experiments causes a rotation of the central tubule complex within the cylinder of nine outer doublet tubules, with this central tubule complex being responsible in part for the coordination of doublet sliding that determines the plane of beat and the direction of flagellar asymmetry. A longitudinal displacement of the central complex by reason of its rotation over the path defined by the helical array of radial spokes that link it to the outer doublets¹³ could then provide the elastic force presumably responsible for spontaneous unwinding. The bridge linking

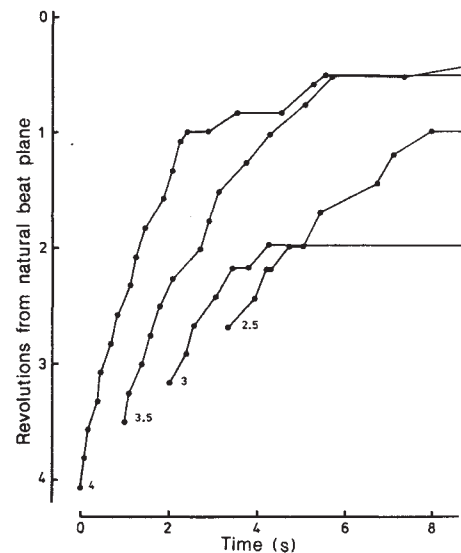


Fig. 3 Time course of the spontaneous unwinding of the beat plane in flagella of four live sperm that have undergone 2.5-4 revolutions of imposed winding, indicated by the number near the bottom of each curve. The small deviations from the corresponding values on the ordinate arise because the natural beat plane (before the vibrator was turned on) did not coincide precisely with the plane of lateral vibration. Starting points of the curves are displaced sideways by arbitrary amounts of clarity. Values of angles between lateral and vertical were determined trigonometrically from the apparent amplitude of the tilted waveform. Vibration frequency and amplitude, 50-54 Hz and $\pm 4.5 \mu\text{m}$, respectively; mean distance from pipette to cover glass, 15-36 μm . Average angular velocity during the first second of unwinding for each sperm was: 4 turns, 10.4 rad s^{-1} ; 3.5 turns, 7.2 rad s^{-1} ; 3.0 turns, 4.6 rad s^{-1} ; 2.5 turns, 3.1 rad s^{-1} .

doublet tubules numbers 5 and 6 (ref. 14) could be responsible for the preferred beat plane revealed by the hesitations that occur during the late stages of spontaneous unwinding, although we cannot exclude the possibility of their being due to hydrodynamic interaction with the glass surface. Study of suppressor mutations that restore motility to paralysed mutants in *Chlamydomonas*¹⁵ has provided genetic evidence linking the functioning of the radial spokes to that of the dynein arms responsible for tubule sliding. Our general hypothesis is testable for it predicts that the effect of larger numbers of forced rotations will differ depending on their direction, with the central pair colliding with the nucleus on rotation in one direction and being extruded from the flagellar tip on rotation in the other. Investigation of the interaction between an imposed vibration and a beating flagellum is a promising approach to elucidating the factors responsible for regulating microtubule-based motility.

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Construction and use of human chromosome jumping libraries from *NotI*-digested DNA

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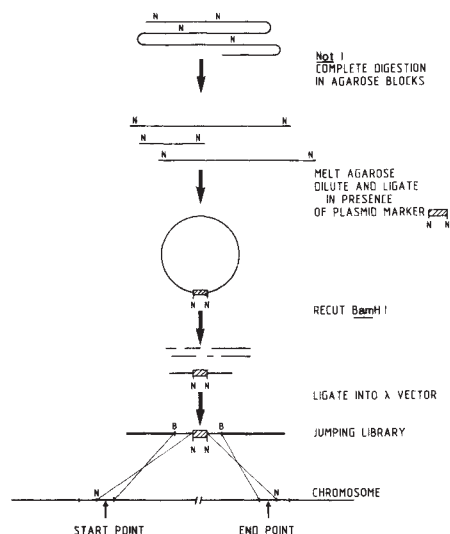


Fig. 1 Schematic drawing of library construction, starting with DNA of large relative molecular mass in agarose blocks.

Methods. Cells of the human cell line LCL127 containing 4X chromosomes were washed once in phosphate-buffered saline (PBS), suspended at a concentration of 1.2×10^7 cells ml^{-1} in 0.5% low gelling temperature agarose (BRL) in PBS, and poured into precooled perspex bar containing 80 μl slots. After cooling, the blocks were incubated at 50°C for 2–4 days in 0.5 M EDTA (pH 7.5), 1% sodium lauroyl sarcosine and 2 mg ml^{-1} Proteinase K. After washing twice for half an hour in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA and 40 μg ml^{-1} phenylmethylsulphonylfluoride (PMSF) at 50°C , blocks were ready for enzymatic digestions or were stored in 0.5 M EDTA (pH 8.0). Blocks were digested for 4 h at 37°C with 16 units *NotI* (NEB) in 200 μl 10 mM Tris-HCl (pH 7.6), 10 mM MgCl_2 , 100 mM NaCl, 500 μg ml^{-1} bovine serum albumin (BRL) and 5 mM spermidine. After digestion the block was washed twice in 10 mM Tris-HCl (pH 8.0) 50 mM EDTA, the enzyme was inactivated in 200 μl of the same buffer containing 200 μg ml^{-1} of Proteinase K (Merck) for 60 min at 37°C . The blocks were then melted at 68°C for 10 min and the Proteinase K was inactivated by addition of PMSF to 0.4 μg ml^{-1} . The tag plasmid used was a derivative of pMLS12 (ref. 12) with a *NotI* site replacing the *BamHI* site, and an *MluI* site replacing the *SalI* site. Before ligation, this plasmid was cleaved with *NotI*, and treated with calf intestine alkaline phosphatase (CIAP, Boehringer-Mannheim) to block self ligation. Two ligations were carried out: ligation A (high) contained 1 μg DNA at a concentration of 0.4 μg ml^{-1} and plasmid at 0.1 μg ml^{-1} ; ligation B (low) contained 3 μg of DNA at a concentration of 0.2 μg ml^{-1} and plasmid at 0.01 μg ml^{-1} , both in 50 mM Tris-HCl (pH 7.6), 10 mM MgCl_2 and 0.2 mM ATP, and 2000 units ml^{-1} T4 DNA ligase (NEB). After ligation overnight at 15°C , NaCl was added to a final concentration of 100 mM, the ligase was inactivated by heating to 65°C for 15 min and the DNA was digested with 20 units ml^{-1} *BamHI* (NEB) for 2 h at 37°C . After digestion, EDTA (pH 7.6) was added to a final concentration of 20 mM, the enzyme was removed by extraction with phenol/chloroform/isoamylalcohol (25:24:1), carrier transfer RNA was added to 10 μg ml^{-1} and the DNA was ethanol precipitated. Pellets were washed in 70% ethanol, and dissolved to a concentration of 30 μg ml^{-1} in 10 mM Tris-HCl pH 8.0, 1 mM EDTA (TE). After treatment with 0.1 units μg^{-1} CIAP for 30 min at 37°C , trinitroacetic acid was added to a final concentration of 10 mM, and the enzyme was inactivated for 30 min at 65°C . The DNA was again precipitated with ethanol and salt removed by washing with 70% ethanol. DNA from each fraction was then dissolved in 50 and 100 μl TE respectively, and ligated in a total volume of 200 μl (400 μl) to 20(40) μg of $\lambda\text{NM1151-AB}$, an Aam-Bam derivative of the lambda insertion vector NM1151 (ref. 13). The ligation product was packaged *in vitro* using 200 μl ligation mixture, 200 μl sonic extract and 500 μl freeze thaw lysate¹⁴ for 3 h at room temperature. Packaged material purified by a CsCl step gradient⁷, fractions containing phages identified by titration, dialysed against 10 mM Tris-HCl (pH 7.6), 10 mM MgCl_2 , each fraction plated on two 22×22 cm Nunc screening plates using MC1061 (ref. 15) as host. Libraries amplified as described⁷.

A basic difficulty in the molecular analysis of genes identified by mutations in the mammalian genome is the need to cover genetic distances corresponding to several hundred kilobases or more by molecular techniques like chromosome walking. In chromosome jumping^{1–3}, this limitation is overcome by the deletion of all but the extreme ends of large DNA molecules before cloning. We describe here the construction and characterization of a *NotI* 'jumping library' from human DNA. To characterize this library, random clones were analysed by restriction mapping. Clones carrying unique end fragments were characterized further by hybridization to Southern blots of *NotI*-cleaved human DNA separated on pulsed field gradient (PFG) gels^{4–6}. As a first step in a directional walk, the library was screened with a clone containing a *NotI* site cleaved in genomic DNA ('*NotI* linking clone')^{2,3,7} localized to the distal third of the short arm of human chromosome 4 (A.-M.F. & T.P., unpublished data). Starting and end points of two identified clones were positioned within a restriction map covering 850 kilobases.

The development of techniques for the molecular cloning and analysis of DNA sequences has allowed major advances in the analysis of genes and gene complexes in many organisms. Most single genes, ranging in size from a few kilobases (kb) to at most a few hundred kb, can either be contained within single λ or cosmid clones, or can be covered by a limited amount of chromosome walking, well within the range of currently available techniques. Analyses of mammalian gene clusters, which require large scale chromosome walking, often already surpass the capabilities of molecular cloning⁸. The distances of millions of basepairs, which typically separate genetically linked markers or mutations in mammals, will generally not be spanned by conventional cloning techniques.

This difficulty is being overcome by progress in two separate areas: the introduction of PFG gel electrophoresis^{4–6} to separate DNA fragments of hundreds or thousands of kb, and the construction and use of specialized, 'chromosome jumping', libraries, which differ from libraries used for chromosome walking in the manipulations carried out before the cloning step (Fig. 1). Jumps over hundreds of kb are possible, since the length of jumps (in contrast to the steps in chromosome walking) is not limited by the capacity of the vector system used.

In library construction^{1–3} large DNA fragments, usually created by partial or complete digestion with restriction endonucleases leaving unique, protruding ends, are circularized at low concentration in the presence of a plasmid, which carries a suppressor transfer RNA gene and has been linearized to generate compatible protruding ends. The concentrations of the long DNA fragments and the plasmid are chosen to give a large fraction of circularization events, in which a chimaeric circle consisting of both plasmid 'tag' and long DNA fragment is formed. The circle is then cleaved at many points by a restriction enzyme not cleaving within the tag plasmid, the DNA fragments are ligated into a λ vector carrying multiple amber mutations, and clones of fragments containing the tag sequence are selected by plating on a host unable to suppress the amber mutations carried by the λ vector.

Though partial digestion with restriction enzymes cutting often in the genome can be used in this protocol, we have

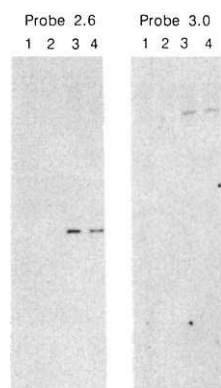


Fig. 2 Mapping of start fragments, and one end fragment, by somatic cell hybrids. Probes 2.6 (start) and 3.0 (end point of longer jumping clone) hybridized to a Southern blot of DNAs of cell lines containing: lane 1, human chromosome 5 in a hamster background; lane 2, only hamster chromosomes; lane 3, human chromosome 4 in hamster background; lane 4, HHW693 (ref. 16). The results position both clones to the sequences shared by the cell lines common to lanes 3 and 4, the distal third of 4p.

preferred to use digestion with *NotI* or similar enzymes, which cut only rarely in mammalian DNA, thus drastically reducing the size of the library required to cover the mammalian genome, and allowing easier analysis of jumps by PFG electrophoresis^{2,3}.

As only products of circularization events including a tag plasmid will be recovered, any given concentration of the tag plasmid will favour the recovery of jumping clones from molecules of a specific size. Shorter molecules will tend to circularize preferentially without including the tag sequence, whereas larger molecules will often ligate to different plasmids at each end, and will be blocked from further circularization by the absence of 5' phosphate groups on the plasmids^{2,3}. To take into account the wide distribution of fragment sizes created by complete digestion with *NotI*, the library was constructed in two fractions, using plasmid concentrations differing by a factor ten. In both cases approximately 30,000 recombinants per μg DNA were generated, using packaging extracts giving 5×10^8 – 5×10^9 clones per μg of λ DNA.

Twelve clones from each library fraction (high and low plasmid concentration) were picked for further analysis. Of the 24 clones, 4 contained no plasmid sequences, probably because suppressor-independent phages were formed by recombination between the vector and packaging phage. This background has been removed by introducing an additional Sam7 mutation in the λ vector to form NM1151 AamBamSam, (H.L., unpublished data). Three clones had lost one or both *NotI* sites during the ligation event, while 17 clones (66%) had the expected structure (vector–*BamHI* site–fragment–*NotI* site–plasmid–*NotI* site–fragment–*BamHI* site–vector). Of these clones, six were excluded because the small size (less than 500 base pairs (bp)) of one arm might complicate further analysis. Of the remaining eleven clones, five (three from the high and two from the low concentration library) were selected for further characterization by PFG analysis. (Unexpectedly only three clones were found to be repetitive, possibly indicating a reduced frequency of repetitive sequences close to *NotI* sites.) *BamHI*–*NotI* fragments were subcloned into a derivative of pEMBL18 (ref. 9) carrying a *NotI* site, and used to probe Southern blots of *NotI*-digested human DNA separated by PFG gel electrophoresis. In four out of five cases both end fragments hybridized to *NotI* fragments of the same size, indicating, but not proving, that both fragments were derived from the same *NotI* fragment. Fragment sizes were ~150 kb, 250 kb and 300 kb (high) and 450 kb (low) (data not shown). The second clone

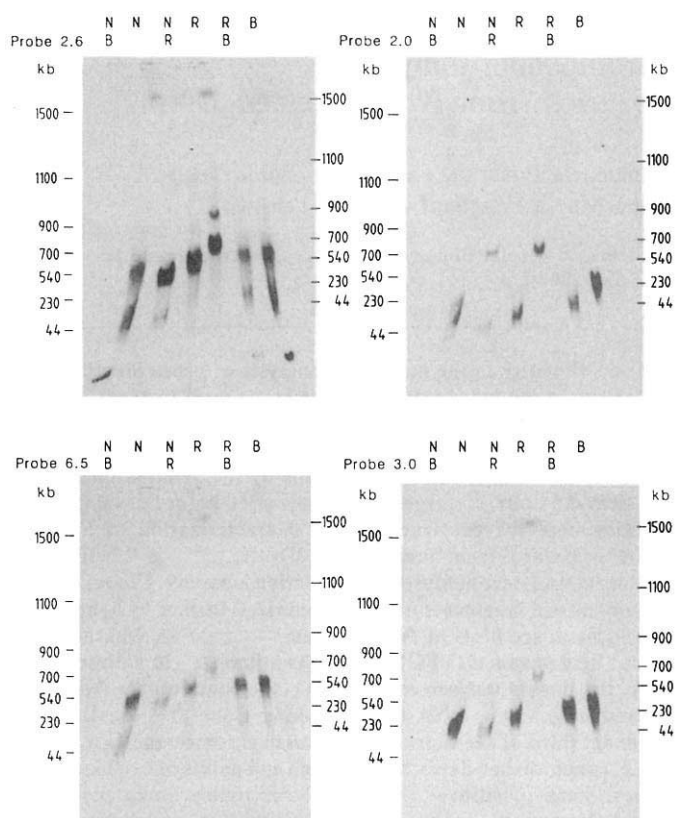


Fig. 3 PFG analysis of clones identified by hybridization to linking clone. Two clones identified by hybridization to one *NotI*–*BamHI* fragment of the linking clone L22 (A.M. & T.P. unpublished data) were isolated, the inserts were subcloned after excision from the phage by *BamHI*, and circularization at a concentration of $1 \mu\text{g ml}^{-1}$. Each end fragment was subcloned as a *BamHI*–*NotI* fragment into a *NotI*-site-containing derivative of pEMBL18 (H.L., unpublished data). The figure shows hybridization of both arms of the linking clone and the two jumping end fragments to a Southern blot of a PFG gel with digests of human DNA (electrophoresis for 36 h at 250 V, 16.5 °C, in a Pulsaphor electrophoresis (LKB) using 120 s pulses). The panel at the top left shows the hybridization pattern with the arm of the linking clone used to screen the jumping library (probe 2.6); top right, hybridization with the other linking clone fragment (probe 2.0). Bottom panels show the hybridization patterns of the same filter with the end points of the shorter (probe 6.5) and longer jumps (probe 3.0) respectively. Lanes from left to right show single and double digests of LCL127 DNA (restriction enzymes: N, *NotI*; B, *Bss*HII; R, *Nru*I). Sizes were assigned taking into account the different migration of size marker lanes (yeast chromosomes and λ multimers) run on both sides of the gel. Redigestion of the *Bss*HII digest with *NotI* (lane 1 from left) was not successful in this particular experiment. This lane therefore shows bands expected from a *Bss*HII digest.

from the low plasmid concentration library hybridized to two different fragments of 800 and 1000 kb with the two end fragments, indicating either the product of an intermolecular ligation event, or, less likely, a very large jump across a partially cleaved *NotI* site.

In general the jumping end points will be located at the ends of the corecognised fragment, allowing a direct determination of the distance jumped from the fragment size, but jumps can occur to internal *NotI* sites cleaved too weakly to be detectable in PFG analysis. This was observed in one clone, which could be shown to represent a jump from the end of a large fragment to a very weakly cleaved internal site (data not shown).

As a first step in a chromosome walk in a defined region of the genome, the *NotI* jumping library was screened with a clone

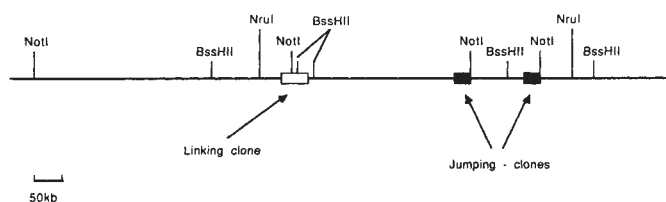


Fig. 4 Localization of jumping end points on a restriction map of the region. The position of start and end points of the two *NotI* jumping clones are placed on a restriction map, covering 850 kb, determined by the analysis of data shown in Fig. 3. The position of the linking clone is drawn as open bar. The jumping clone end points are indicated by filled bars.

selected to contain a *NotI* site cleaved in human DNA (*NotI* linking clone), derived from the distal third of chromosome 4p (A-M.F. & T.P., unpublished data). Two jumping clones extending in the same direction were identified, their end points mapped to the same subregion of human chromosome 4 by somatic cell genetics (Fig. 2), and analysed further by hybridization to PFG Southern blots (Fig. 3). From these results and the analysis of Southern blots of single and double digests of human DNA with the enzymes *NotI*, *BssHII* and *NruI* a restriction map of a 850 kb region containing the linking and jumping clones as derived, and the clones were positioned within the map (Fig. 4). This analysis showed the two clones to be derived from the ends of two adjacent *NotI* fragments of 250 and 100 kb in size, representing jumps of 250 and 350 kb respectively (expected to correspond roughly to 0.25 and 0.35 cM in genetic distance).

Although the use of complete digestion libraries offers many advantages, there are inherent difficulties associated with their use. One complication is the need to start a jump (or a series of jumps) from fragments adjacent to the corresponding rare cutting site. If no such sites are available close to the starting point, such fragments can be selectively cloned either by using special jumping libraries allowing jumps towards *NotI* sites^{2,3} or the selective cloning of ends from *NotI* fragments purified by pulsed field gel electrophoresis (F. M. Michiels, M. B. Burmeister and H.L., in preparation). Complications can be expected due to the existence of *NotI* fragments too large to circularize, or the occurrence of *NotI*-*Bam*HI fragments too large to be cloned in the vector used.

Both problems can be reduced considerably by connection between jumping libraries prepared using different rare cutting enzymes. This is often possible due to clustering of many rare cutting sites in 'HTF islands'^{10,11}. The use of alternative enzymes in the recleavage reaction or vectors with larger capacity will also reduce problems with end points found unclonable due to their size in one library.

We have shown the construction and characterization of a human *NotI* jumping library and its use in the analysis of a region of human chromosome 4, defined by a *NotI* linking clone isolated from that region. The construction of jumping libraries using enzymes cutting rarely in the mammalian genome reduces the complexity of the libraries to be constructed and screened. The characterization of the resulting clones is simplified, and, due to the often observed clustering of rare-cutter restriction sites within HTF islands¹⁰, close to genes¹¹, tends to give immediate access to transcribed regions. These libraries also provide a connection between molecular cloning, genetic analysis and physical mapping by PFG electrophoresis. The combination of jumping and linking libraries offers the possibility of directional walking, and might allow the analysis of longer regions in parallel mapping strategies³.

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background. We also thank Marija Bucan and Margit Burmeister for providing Southern blot filters for PFG gel analysis, and Frank Michiels for suggestions on the manuscript. The work was partly supported by the Hereditary Disease Foundation.

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DNA ligase I deficiency in Bloom's syndrome

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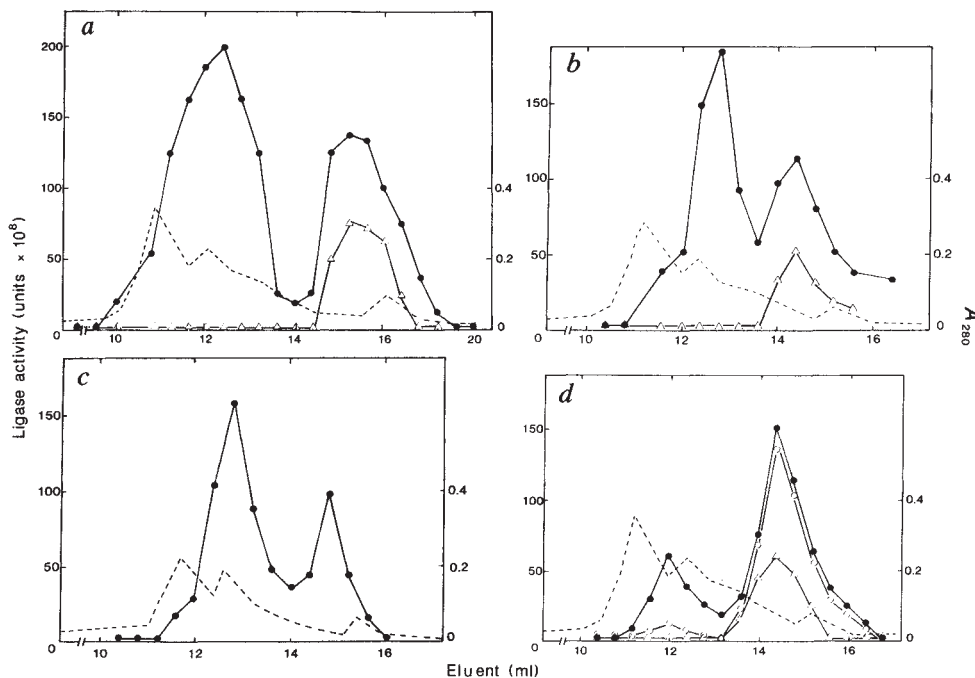
Certain rare human diseases with autosomal recessive mode of inheritance are associated with a greatly increased cancer frequency which may reflect specific defects in DNA repair or replication. These disorders include xeroderma pigmentosum, ataxia-telangiectasia, Fanconi's anaemia and Bloom's syndrome¹. Cells from individuals with Bloom's syndrome² usually grow slowly in culture and exhibit increased chromosomal breakage and rearrangement, an elevated frequency of sister chromatid exchanges, retarded rates of progression of DNA replication forks, delayed conversion of replication intermediates to high-molecular-weight DNA, and slightly increased sensitivity to DNA-damaging agents³⁻¹¹. Several of these features are also characteristic of *Escherichia coli* and yeast mutants with a defective DNA ligase¹²⁻²². In this investigation we show that one of the two DNA ligases of human cells, ligase I, is defective in a representative lymphoid cell line of Bloom's syndrome origin.

Mammalian cells, in contrast to bacteria and yeast, have two different DNA ligases, which we have designated ligase I and ligase II (refs 23-25). Both enzymes are present in the cell nuclei but they do not cross-react serologically²³⁻²⁸. The larger enzyme, DNA ligase I, is induced during cell proliferation and may be active in chromosomal replication, whereas the smaller enzyme, ligase II, is present in similar amounts in growing and non-growing cells. Recently, selective assay procedures have been devised for these enzymes to facilitate their characterization²⁹. Thus, only ligase I can catalyse the joining of blunt-ended DNA fragments, whereas ligase II can link oligo(dT) molecules, annealed to poly(rA).

The levels of DNA ligases I and II were measured in human lymphoid B-cell lines (from the Human Genetic Mutant Cell Repository, Camden, New Jersey) representative of different syndromes. Extracts of the cells were size-fractionated by liquid chromatography and assayed for total DNA ligase activity using a poly(dA)·oligo 5'-³²P-(dT) substrate^{29,30}. In control cell lines, a large peak of DNA ligase I of relative molecular mass 200,000 (*M*_r 200K) and a smaller peak of ligase II (*M*_r 80K) were

Fig. 1 DNA ligase activities in human lymphoid cell lines representative of different human inherited syndromes with possible DNA replication or repair defects. ●, Assays with poly(dA)·oligo(dT) substrate, measuring both DNA ligase I and ligase II; ○, the same assay carried out in the presence of antiserum against ligase I (0.25 µg NH₄)₂SO₄-fractionated antiserum²³ per reaction mixture); △, results with a poly(rA)·oligo(dT) substrate, measuring specifically DNA ligase II (ref. 29); dashed line, A₂₈₀. The cell lines analysed were: *a*, Raji, a Burkitt's lymphoma line; *b*, GM2250, xeroderma pigmentosum, complementation group A; *c*, GM1526, ataxia-telangiectasia; *d*, GM3403, Bloom's syndrome.

Methods. Cells were grown in RPMI 1640 medium supplemented with 15% fetal calf serum and antibiotics. The cells (5×10^7 – 1×10^8) were collected by centrifugation, washed twice in phosphate-buffered saline, re-suspended in 300 µl ice-cold extraction buffer (0.1 M NaCl, 50 mM Tris-HCl, pH 7.5, 10 mM 2-mercaptoethanol, 1 mM EDTA, 23 µg ml⁻¹ aprotinin, 0.5 µg ml⁻¹ each of pepstatin, leupeptin and chymostatin) and disrupted in a glass hand homogenizer. After 1 h at 0°C, debris was removed by centrifugation for 10 min in an Eppendorf microfuge, and the crude cell extract supplemented with 0.05 volume each 2 M NaCl and 10% Polymin-P to precipitate nucleic acids. After 30 min at 0°C, the material was centrifuged and the supernatant applied to an FPLC Superose-12 column (Pharmacia) equilibrated with 50 mM NaCl, 50 mM Tris-HCl (pH 7.5), 10 mM 2-mercaptoethanol, 1 mM EDTA. The column was eluted at 0.4 ml min⁻¹ 200 µl fractions were collected, and DNA ligase assays were performed on 50 µl aliquots in a final reaction mixture volume of 60 µl.



observed. Typical data are shown in Fig. 1*a*. These peaks represent the active monomers^{26,27} of DNA ligases I and II. Under these extraction and fractionation conditions, no dimers²⁸ of DNA ligase I were observed. That the second peak of activity was DNA ligase II was confirmed by employing a poly(rA)·oligo(dT) substrate²⁹ (Fig. 1*a*). Elution profiles not significantly different from those of control cells were seen with extracts from lines GM2250 (xeroderma pigmentosum, complementation group A) (Fig. 1*b*), GM4510 (Fanconi's anaemia, data not shown) and GM1526 (ataxia-telangiectasia) (Fig. 1*c*). We conclude that there is no general deficiency in DNA ligase activity in cells representative of human chromosome breakage syndromes.

When the Bloom's syndrome cell line GM3403 was investigated, a reduced amount of DNA ligase I activity was observed (Fig. 1*d*), whereas the level of DNA ligase II was similar to that seen with control cells. This lymphoid cell line was derived from a confirmed case of Bloom's syndrome of Ashkenazi origin by the Human Genetic Mutant Cell Repository in collaboration with Dr K. H. Kraemer (NCI); it has retained the properties of high sister chromatid exchange and chromosome instability characteristic of the disease. The residual activity eluting at the expected position of ligase I in the GM3403 line was inhibited by 80% using rabbit antibodies²³ against DNA ligase I (Fig. 1*d*), which confirms that this high *M_r* peak of DNA ligase represented a small amount of active DNA ligase I. Furthermore, this fraction showed significant ability to perform blunt-end ligation of DNA, when assayed as described²⁹. Thus the GM3403 cells, although not totally deficient in ligase I, contain one quarter of the activity found in other lymphoid lines.

The reduction in DNA ligase I activity in the Bloom's syndrome line could be due to a regulatory mutation, or to a structural defect in the enzyme itself. To distinguish between these possibilities, we determined the heat lability of the partly purified, size-fractionated DNA ligase I from the GM3403 line as well as from control cell lines (GM2250 and Raji). Figure 2*a*

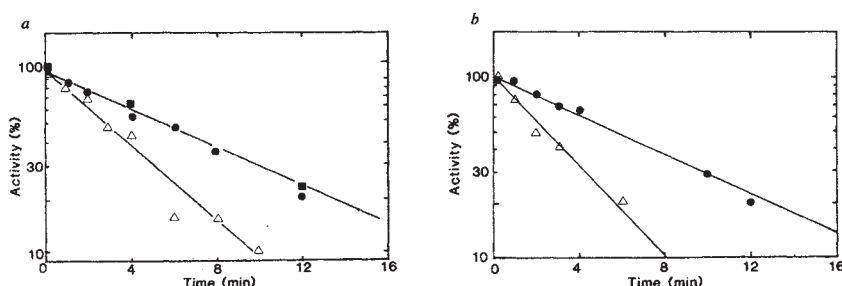
shows that the Bloom's syndrome line contains a DNA ligase I that is more heat-labile than the enzyme from control cells. On further purification of ligase I from the lines GM3403 and Raji by DNA-cellulose chromatography, the relative heat-sensitivity of the Bloom's syndrome enzyme persisted in comparison with the control enzyme (Fig. 2*b*). In contrast, DNA ligase II from the Bloom's syndrome cells showed the same heat lability (50% inactivation in 5 min at 42°C) as ligase II from control cells (data not shown).

The GM3403 cell line is the only representative Bloom's syndrome lymphoid line available from a mutant cell culture collection: experiments with the fibroblast line GM5289, derived from a Japanese case of Bloom's syndrome, showed that DNA ligase I from this source was not obviously heat-sensitive or present in reduced amounts. However, in contrast to the enzyme from lymphoid cells (Fig. 1) or from HeLa cells, the GM5289 DNA ligase I eluted as a mixture of 30% dimers²⁸ and 70% monomers on size fractionation by liquid chromatography (data not shown). Altered aggregation properties of DNA ligase I in Bloom's syndrome cells have also been observed by Chan *et al.*³¹.

Certain cell lines derived from Bloom's syndrome appear to represent revertants that no longer exhibit high sister chromatid exchange or other typical features of cells from patients^{32,33}. For example cells from the lymphoid cell line GM4408 (Human Genetic Cell Repository) do not show elevated sister chromatid exchange and we have found normal levels of both DNA ligases I and II in cell extracts (data not shown). An interesting example of a strain with altered properties is the fibroblast line GM1492, which differs from other Bloom's syndrome fibroblasts in growing well in culture and having a preneoplastic phenotype³⁴; these cells contain high levels of a presumably recombinogenic protein similar to the *E. coli* RecA protein³⁵ and a factor which appears to promote DNA ligase activity (S. Ljungquist, personal communication).

There is no evidence for multiple genetic complementation groups in Bloom's syndrome, and this inherited disease may

Fig. 2 Heat lability of DNA ligase I from a Bloom's syndrome cell line at different stages of enzyme purification. *a*, Pooled peak fractions (0.1–0.2 mg protein per ml) of the size-fractionated DNA ligase I (Fig. 1) were incubated at 50 °C, and aliquots removed at different times for ligase assays. ●, Ligase I from Raji cells; ■, ligase I from xeroderma pigmentosum group A; △, ligase I from Bloom's syndrome. *b*, In separate 10-fold scaled-up experiments, the Bloom's syndrome GM3403 line was propagated as 21 suspension cultures in 31 spinner vessels under conditions that allowed a growth rate similar to that observed for cell lines representative of other human syndromes: ●, △, as in *a*. DNA ligase I from Raji and GM3403 cells was purified by gel filtration on AcA-34 columns (LKB, Inc.) to obtain results similar to those shown in Fig. 1*a* and *d*. Pooled peaks of DNA ligase I activity were dialysed separately against 50 mM Tris-HCl (pH 7.5)/5 mM KCl, 10 mM 2-mercaptoethanol, 1 mM EDTA, and applied to columns of double-stranded DNA-cellulose⁴³. These columns were eluted stepwise with the same buffer containing 50, 100 and 200 mM KCl. DNA ligase I was eluted at 100 mM KCl in both cases, and the enzyme was ~20-fold further purified by this procedure. The pooled active fractions of DNA ligase I (15 µg protein per ml) were incubated at 50 °C and assayed for ligase activity as in *a*.



well be due to a single defective polypeptide. Our results suggest that this protein is identical to DNA ligase I. Note that DNA ligase II (this work) as well as DNA polymerases α , β and γ (refs 36, 37) are normal in Bloom's syndrome cells. A number of different possible explanations for the cellular defect in Bloom's syndrome have been advanced previously, such as the release of a clastogenic factor or altered regulation of the synthesis of DNA repair enzymes^{38–42}, but these theories do not seem to account for the observed phenotypic changes. In contrast, a DNA ligase deficiency could explain the increased frequency of sister chromatid exchanges, because retarded joining of DNA strand interruptions would be expected to promote homologous recombination events. Furthermore, a defective DNA ligase could account for the anomalously slow conversion of DNA replication intermediates to a high M_r form. Because ligase I is likely to be required for DNA replication, presumably only leaky mutants can survive, and the variable phenotype of different cellular strains is probably related to different amounts of residual enzyme activity. The apparent relationship of Bloom's syndrome to an alteration in the structural gene for DNA ligase I makes the molecular cloning of this gene a priority, and suggests experimental strategies for the detection of aberrant DNA sequences in heterozygotes.

Since submission of this manuscript, a second Bloom's syndrome lymphoblastoid cell line (obtained from Dr E. Henderson, Temple University School of Medicine, Philadelphia) has also been found to contain reduced amounts of ligase I, and the enzyme is likewise anomalously heat-sensitive.

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Altered DNA ligase I activity in Bloom's syndrome cells

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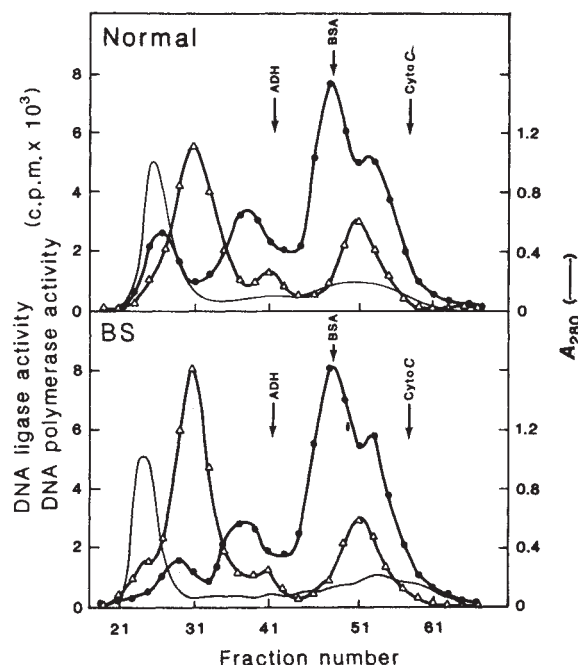
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Cells from patients with Bloom's syndrome, a rare disease associated with increased cancer frequency, exhibit cytological abnormalities^{1–3}. These include increased numbers of homologous chromatid interchange figures and sister-chromatid exchanges, together with abnormally slow replicon-fork progression^{4–6} and retarded rate of DNA-chain maturation^{7,8}, and suggest that the primary defect in this recessive disorder affects S-phase DNA replication. DNA ligases and DNA polymerases have long been prime candidates for abnormality in Bloom's syndrome, but various studies of DNA polymerases in Bloom's syndrome cells have disclosed no abnormalities^{9–11}. Evidence is presented here, as in the accompanying paper from a different laboratory¹², for the existence in Bloom's syndrome of an abnormality of the DNA ligase involved in semi-conservative DNA replication.

DNA ligase I and DNA polymerase α are enzymes that function during DNA replication; DNA ligase II and DNA polymerase β function during DNA repair^{13–19}. Size heterogeneity has been reported for DNA ligase I (refs 13–15,

Fig. 1 Aca34 gel-filtration elution profiles of DNA ligases and DNA polymerases, comparing normal (HG 1125) and BS (HG 1270) cells. Top panel, normal cell line HG 1125; lower panel, BS cell line HG 1270. ●, DNA ligase activity; △, DNA polymerase activity; continuous line, absorbancy at 280 nm. Data from experiment 1.

Methods. The Epstein-Barr-virus-transformed lymphoblastoid cell lines studied were developed as clones in collaboration with Dr E. Henderson, Temple University, Philadelphia, using the limiting dilution method²². Data from the analysis of extracts from cell lines HG 1125, derived from a normal woman, and HG 1270, derived from a boy with BS (identified in the Bloom's Syndrome Registry as 15[MaRo]), are presented in Fig. 1. In a second experiment (elution profiles not shown), extracts prepared from cell lines HG 1522, derived from a normal man, and HG 1525, derived from a baby boy with BS (identified in the Bloom's Syndrome Registry as 372[MaGrou]), were used. The cells were cultured in spinner flasks in RPMI 1640 medium supplemented with 20% heat-inactivated fetal bovine serum, 100 IU ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin, and 2 mM L-glutamine. The cells were pelleted, washed once with Dulbecco's phosphate-buffered saline (PBS), and frozen in PBS containing 10% glycerol (-70 °C) until a total of 1–2 × 10⁸ viable cells from each line had been accumulated. Cell extracts were prepared and assayed as described^{20,23}. The accumulated cells were thawed, homogenized in extraction buffer (0.05 M Tris-HCl [pH 8.0], 2.0 M NaCl, 0.1 mM EDTA, 2.5 mM MgCl₂, 0.5% Triton X-100, 1 mM dithiothreitol (DTT), 5 mM β-mercaptoethanol, 0.1 mM phenylmethylsulphonyl fluoride), and sonicated using a Branson sonicator (20 s). The cell extract was centrifuged (105,000g, 60 min), and the supernatant was dialysed against TGEDM buffer (0.02 M Tris-HCl [pH 8.0], 10% glycerol, 0.1 mM EDTA, 1 mM DTT, and 5 mM β-mercaptoethanol) containing 0.8 M NaCl (2–4 h; 4 °C). Equal amounts of extracted protein from the different cell lines were loaded onto separate Aca34 gel-filtration columns (90 cm × 1 cm, LKB, Bromma, Sweden) and eluted as fractions (3 ml) using TGEDM buffer containing 0.8 M NaCl as the eluant. The fractions were dialysed against TGEDM buffer and assayed for DNA ligase and DNA polymerase activity. Yeast alcohol dehydrogenase (151K), bovine serum albumin (68K), and cytochrome c (12K) were used as standards. *M_r* values for eluates were estimated using the technique of Andrews²⁴. DNA ligase activity was assayed as described by Modrich and Lehman²⁵, using [³H]-poly (dA-dT) as a template. The reaction mixture contained 50 mM Tris-HCl (pH 8.0), 5 mM MgCl₂, 0.1 mM Na₂-EDTA, 0.2 mM ATP, 4 mM DTT, 80 µM [³H]-poly(dA-dT) (specific activity, 1020 c.p.m. pmol⁻¹, Miles Laboratories), and enzyme extract in a final volume of 0.4 ml. After incubation (30 °C, 30 min), the reaction was terminated by heating the mixture (90 °C, 5 min) and chilling it on ice (10 min). Exonuclease III (25 units, BRL) then was added, and the mixture was re-incubated (37 °C, 60 min). The reaction was terminated by chilling and adding 1 ml trichloroacetic acid (TCA) containing 100 µg yeast RNA. The samples were filtered through GF/C glass-fibre filters (Whatman); the filters were washed with 5% TCA, soaked in 5 ml Scintiverse (Fisher), and counted in a liquid scintillation counter. One unit of ligase activity was defined as 1 pmole of [³H]poly(dA-dT) resistant to exonuclease III digestion after incubation at 30 °C for 30 min. DNA polymerase activities were assayed as described by Chan and Becker²⁶. The reaction mixture contained 50 mM Tris-HCl (pH 7.4), 6 mM magnesium acetate, 30 mM DTT, 50 µg ml⁻¹ activated calf thymus DNA, 100 µM dATP, dCTP, and dGTP, 20 µM dTTP, 1 µCi [³H]dTTP, (specific activity 170 c.p.m. pmol⁻¹, ICN), and enzyme extract in a final volume of 0.2 ml. DNA synthesis was measured by the incorporation of [³H]dTTP into TCA-insoluble material.



Three peaks of DNA ligase activity, designated DNA ligases Ia, Ib, and II in order of their elution from the column, were detected in extracts from both types of cells (Fig. 1). The elution profiles of DNA ligases Ib and II from normal and BS cells were similar (estimated relative molecular mass of 240,000 and 80,000 respectively, *M_r* 240K and 80K); in the first experiment, however, the elution profiles of DNA ligase Ia from the normal (HG 1125) and BS (HG 1270) cells differed: DNA ligase Ia from cell line HG 1270 eluted from the column in fractions that would

18, 19), but the physiological relationship between the multiple forms is unknown. DNA ligase Ia and Ib activities are increased significantly in rapidly proliferating tissues whereas DNA ligase II activity remains at baseline level in such tissues²⁰. DNA ligase II activity is stimulated by DNA-damaging agents.

In two separate experiments, extracts from normal and Bloom's syndrome (BS) cells were chromatographed on Aca34 gel-filtration columns, and the column fractions were assayed for both DNA ligase and DNA polymerase activity (Fig. 1).

Table 1 DNA ligase and DNA polymerase activities in Bloom's syndrome (BS) and control normal (N) B-lymphoblastoid cell lines

Enzyme	BS (HG 1270)	Specific activity (pmol per mg protein)			BS (HG 1525)	N (HG 1522)	P
		Experiment 1 N (HG 1125)	P				
Ligase Ia	8.4 ± 2.2 (52)	16.2 ± 3.5	<0.02		8.2 ± 1.7 (45)	18.2 ± 2.8	<0.05
Ligase Ib	23.8 ± 3.8 (87)	27.4 ± 4.6	<0.20		82.0 ± 9.3 (98)	83.6 ± 33.3	<0.50
Ligase II	84.0 ± 8.6 (109)	77.5 ± 10.5	<0.25		94.8 ± 31.2 (85)	115.5 ± 20.9	<0.30
Polymerase α	701.0 ± 85.4 (126)	555.0 ± 54.1	<0.05		1005.0 ± 119.4 (121)	830.0 ± 25.9	<0.20
Polymerase β	260.0 ± 38.0 (107)	242.0 ± 34.7	<0.30		555.0 ± 56.7 (230)	241.0 ± 63.6	<0.025

Values shown as mean specific activity ± s.d. represent the average of three separate determinations in Experiment 1 and two in Experiment 2. Values in parentheses represent the enzyme specific activity in BS cells as a percentage of that in normal (N) cells. Values of *P* were derived from the Student *t*-test.

be expected for a 400K protein rather than the expected 480K that was observed for DNA ligase Ia from normal cells. Information concerning this suggested difference was not obtained in the second experiment because of poor resolution of the peaks of DNA ligase Ia activity (elution profiles not shown). The DNA ligase II peaks from both normal and BS cells exhibited a low-molecular-weight shoulder of activity, suggesting that multiple DNA ligase II species exist. DNA polymerases α and β from normal and BS cells exhibited similar elution profiles, polymerase α eluting as major and minor peaks of activity of 320–360K and 150–170K, polymerase β eluting as a 40K peak.

DNA ligase Ia activity in both of the BS cell lines that were studied was reduced significantly in comparison to normal ($P < 0.02$ and $P < 0.05$, Table 1). DNA ligase Ib and II activities were similar to those of normal cells. DNA polymerase α activities were somewhat elevated above normal in both BS cell lines; however, the differences were not statistically significant in experiment 2 ($P < 0.20$). DNA polymerase β activities in the two BS cell lines were variable, being normal in one instance (HG 1270) and elevated twofold in the other (HG 1525). The basis for the variability of DNA polymerase β activity in BS cells is unknown, but similar variabilities have been detected in another laboratory¹⁰.

Given the normal levels of DNA polymerase α activity in the two BS cell lines, it is unlikely that the significantly lower DNA ligase Ia activity demonstrated in those same lines resulted from slow BS cellular proliferation. Furthermore, growth studies performed earlier (ref. 21 and unpublished data) demonstrated that the normal and BS cell lines used in these experiments exhibit similar doubling times.

The finding of abnormal DNA ligase Ia activity in BS cells is consonant with cytogenetic^{1–3} and biochemical^{4–11} data derived from studies of BS cells. Nevertheless, the question as to whether abnormal DNA ligase Ia activity is the primary biochemical defect in BS cells remains unanswered. Even though the DNA ligase Ia alteration reported here may be responsible for certain aspects of the BS phenotype, further study is required to rule out the possibility that the demonstrated DNA ligase Ia deficiency is secondary to some other metabolic disturbance. Confirmation that the physico-chemical features of DNA ligase Ia from BS cells differ from normal, as is indicated by the results of one of the two experiments reported here, would constitute proof that DNA ligase Ia truly is the primary biochemical defect in BS.

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Elevated levels of diacylglycerol and decreased phorbol ester sensitivity in *ras*-transformed fibroblasts

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Diacylglycerol (DG) plays a central role in phospholipid metabolism and is an endogenous activator of protein kinase C (refs 1–3). We have suggested^{4,5} that constitutive activation of this kinase is one mechanism by which oncogenes transform cells. The *ras*-encoded proteins are similar to regulatory G-proteins⁶ and are candidates for the unknown G-protein that modulates phosphatidylinositol (PI) turnover^{1,7,8}. Differences in polyphosphoinositide metabolism have been reported for *ras*-transformed cells⁹. But because these experiments were performed on confluent cultures of established cell lines, the differences are difficult to attribute to *ras* transformation. Here we show that exponentially growing NIH 3T3 fibroblasts recently transformed by Ha-*ras* or Ki-*ras* possess elevated DG concentrations without significant alterations in the levels of other polyphosphoinositide metabolites. The basal phosphorylation of protein kinase C substrate of relative molecular mass (M_r) 80,000 (80K) is significantly increased in all the *ras*-transformed cell lines. Surprisingly, however, further phosphorylation of this protein on addition of phorbol ester was greatly reduced. Ha-*ras* cells also show less binding of phorbol ester than control cells, suggesting that elevation of DG causes partial down-regulation in addition to activation of protein kinase C.

The effects of transformation by *ras* on PI metabolism were investigated in NIH 3T3 cells recently transformed either by transfection with activated Ha-*ras* or by infection with Kirsten sarcoma virus, to avoid artefactual changes resulting from long-term cell-line divergence. Steady-state levels of phosphoinosi-

Table 1 Steady-state levels of [5,6,8,9,11,12,14,15-³H]-arachidonate-labelled DG and of [2-³H]-inositol-labelled inositol polyphosphates

Cell type	³ H-d.p.m. (normalized)	% total ³ H-phosphoinositides		
	DG	1,3,4-InsP ₃	1,4,5-InsP ₃	InsP ₄
NIH 3T3	1,661 ±129 (3)	0.0052 ±0.0009 (4)	0.0083 ±0.0047 (4)	0.0038 ±0.0012 (4)
Ha 1-3T3	4,627 ±138 (3)*	0.0048 ±0.0005 (4)	0.0093 ±0.0037 (4)	0.0051 ±0.0001 (4)

NIH 3T3 cells transfected with a pBR322 plasmid containing a simian virus 40 (SV40) promoter and the activated Ha-*ras* oncogene from the T24 bladder carcinoma cell line. Foci of transformed cells were expanded and frozen for storage in liquid nitrogen within two weeks. Expression of Ha-*ras* was tested by Western blotting of normal and transfected cell lysates using an anti-p21^{ras} monoclonal antibody Y13-259 (R. Stein, Meck Sharpe and Dohme). Cultures were labelled to isotopic equilibrium with 1 μ Ci ml⁻¹ (5,6,8,9,11,12,14,15-³H)-arachidonic acid (155 Ci mmol⁻¹; Amersham) and 0.5 μ Ci ml⁻¹ ³²P₄, or 5 μ Ci ml⁻¹ [2-³H]-myo-inositol. ³H-DG was normalized to 10,000 d.p.m. ³²P-phospholipid. Because all the ³²P₄ remained at the origin during TLC, it was important to ensure that the radioactivity was actually in phospholipids rather than in aqueous contamination. Therefore, samples were also developed in a chloroform/methanol/NH₄OH/water system⁵. In this solvent system all the ³²P₄ radioactivity ran up from the origin, indicating that it was incorporated into phospholipid. For analysis of inositol phosphates, cells were extracted and processed as described by Downes and Michell¹⁷ and samples were analysed by HPLC essentially as described¹⁰. Typically, 1–4,000 ³H d.p.m. were associated with each species. These data were normalized to total ³H-labelled phosphoinositide present in corresponding lipid extracts, prepared as described by Downes and Michell¹⁷.

* $P < 0.001$.

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tides and inositol phosphates were measured on Ha-3T3 and wild-type 3T3 cells labelled to isotopic equilibrium with [^3H]myo-inositol under subconfluent conditions. No significant differences were detected in the ^3H -phosphoinositide concentrations between the control and transformed cell lines (data not shown). Analysis of the inositol phosphates was performed by high pressure liquid chromatography (HPLC) to separate the recently discovered inositol-tetrakisphosphate (InsP_4) and the 1,3,4- and 1,4,5-isomers of inositol-trisphosphate (InsP_3) (ref. 10). No significant differences were apparent between the Ha-*ras*-transformed cells and the control cells (Table 1). Treatment of the transformed cells with the inositol phosphate (InsP) phosphatase inhibitor LiCl at 20 mM for 30 min before extraction did not elevate the level of InsP (data not shown), indicating that phosphoinositide turnover is not dramatically increased by *ras*-transformation, although it remains possible that a minor phospholipid pool is affected by the activated *ras* oncogene.

We then measured the cellular concentrations of DG labelled with [^3H]glycerol to isotopic equilibrium. DG has been implicated in the maintenance of the transformed phenotype of erythroleukaemia cells¹⁶, and the changes in this lipid observed during erythroleukaemia cell differentiation were much greater than those in other PI metabolites¹⁶. Every cell line examined had significantly elevated DG levels compared with the wild type (Table 2), and the level of ^3H -DG in an NRK cell line transformed by Kirsten sarcoma virus was also higher than that of the parent NRK line. The increases (25–110%) are within the range observed on stimulation of 3T3 cells with mitogens such as platelet-derived growth factor¹¹ or prostaglandin $\text{F}_{2\alpha}$ (ref. 12). As these measurements reflect total cellular DG, and the physiological DG pool relevant to kinase C activation probably consists of 2-arachidonate-DG localized to the plasma membrane¹³, the full extent of the differences between control and transformed cells is probably masked by the remainder of the cellular DG. Indeed, we found that differences between control and Ha-*ras*-transformed cells were substantially larger for ^3H -arachidonate-labelled DG than for total cellular ^3H -glycerol-DG (Table 1). These results suggest that a specific perturbation in lipid metabolism accompanies transformation by *ras* oncogenes.

To determine if this perturbation is of functional significance, we examined the responsiveness of the cell lines to phorbol esters, which can mimic the activation of protein kinase C by DG (ref. 3). As a marker of kinase C activation, we observed the phosphorylation in intact cells of an 80K protein that is a ubiquitous substrate for the kinase¹⁴. Untreated control cells

Table 2 Steady-state (^3H -glycerol)-DG levels in *ras*-transformed cells

Cell type	% DG	% of control
NIH 3T3 ($n=2$)	5.29 ± 0.15	100
Ha 1-3T3 ($n=2$)	6.63 ± 0.03	125
Ha 2-3T3 ($n=2$)	7.49 ± 0.43	142
Ha 3-3T3 ($n=2$)	7.08 ± 0.14	134
KSV-3T3 ($n=2$)	11.08 ± 1.10	210
NRK	4.0	100
KSV-NRK	7.4	185

Methods as in Table 1 except for the following. NIH 3T3 cells transformed by infection with Kirsten sarcoma virus (KSV). After incubation in Dulbecco's modified Eagle's medium (DMEM) with $0.4 \mu\text{g ml}^{-1}$ polybrene (Sigma), overnight fresh media with $0.4 \mu\text{g ml}^{-1}$ polybrene and KSV was added. After 1 h at 37°C this was replaced with normal DMEM. 10–14 days later, foci began to form and were picked off and grown in soft agar plates. Cells isolated from the soft agar were tested for elevated levels of *ras* by immunoblotting with Y13-259 and immediately frozen in liquid nitrogen. Thawed cells were not grown for more than two months in culture. The NRK cell lines were a gift from Robert Stein. Cultures were labelled to isotopic equilibrium with $5 \mu\text{Ci ml}^{-1}$ [^3H]-glycerol. ^3H -DG was extracted and analysed by TLC as described¹⁶, normalizing to total ^3H -labelled lipids.

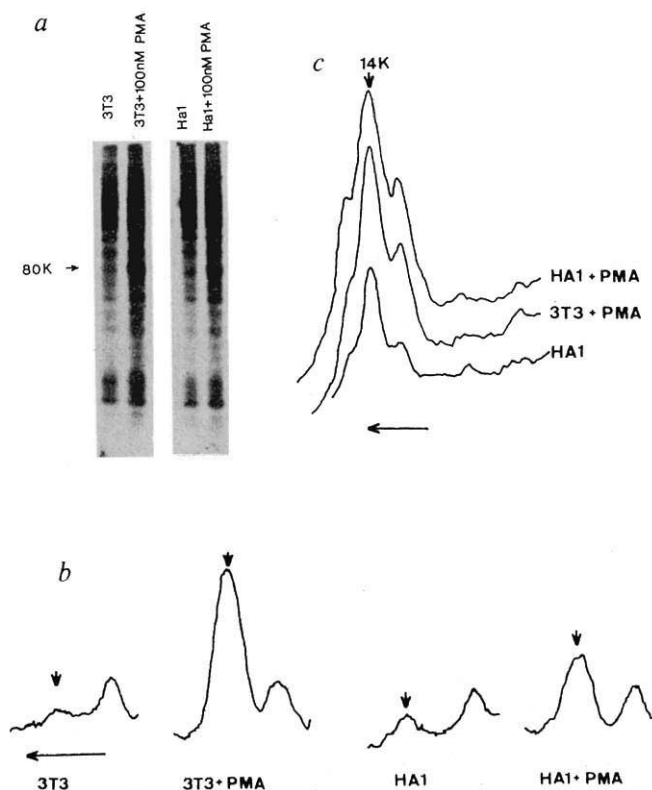


Fig. 1 Quantitation of 80K protein phosphorylation. *a*, Autoradiography of cell lysates; *b*, densitometry of 80K region of *a*; *c*, proteolytic mapping of 80K protein. *a*, Exponentially growing cultures in 35-mm dishes were washed once with phosphate-free HEPES-buffered saline then incubated in phosphate-free MEM (Flow Labs) with $100 \mu\text{Ci ml}^{-1}$ $^{32}\text{P}_4$ and 1 mg ml^{-1} bovine serum albumin for at least 1 h (37°C). Phorbol ester was added from a freshly prepared stock solution to a concentration of 200 nM and after incubating at 37°C for 5 min, the medium was aspirated off and the dishes were immersed into three consecutive 250-ml solutions of ice-cold phosphate-buffered saline (PBS). Trichloroacetic acid (TCA; 10%) was then added and after 30 min at 4°C , the TCA solution was aspirated off, the cell debris rapidly washed three times with 5 ml ice-cold 20 mM phosphate, pH 7.6, and $150\text{--}200 \mu\text{l}$ of 100°C Laemmli sample buffer¹⁸ was added. After transfer to microfuge tubes and boiling for 20 min the samples were normalized to total acid-insoluble ^{32}P (phospholipid plus nucleic acid plus protein) and analysed by 10% SDS-polyacrylamide gel electrophoresis (PAGE). The gels were stained with Coomassie, destained and autoradiographed (*a*) using preflashed Kodak XAR film. *b*, Densitometer scans of the 80K region of the autoradiograph, shown in *a*, using a LKB Laser Ultrascanner. Arrows indicate the position of the 80K band. Background was subtracted after calculating the mean minimum intensity during an initial scan of each lane. *c*, Proteolytic mapping of the 80K phosphoprotein was performed as described by Cleveland *et al.*¹⁹, using $0.5 \mu\text{g}$ V8 protease per well and 15% SDS-PAGE gels. Autoradiographs of the gels were scanned by densitometry as in *b*.

showed very low levels of phosphorylation of this protein. On stimulation with phorbol 12-myristate, 13-acetate (PMA) or phorbol dibutyrate (PDBu) however, a dramatic increase in phosphorylation occurred (Fig. 1*a, b*, Table 3). The basal level phosphorylation of the 80K protein in the *ras*-transformed cell was 4–20-fold higher, and addition of PMA caused only a small further increase in phosphorylation, to a level considerably lower than that in the control cells. Analysis of the phosphoproteins by Cleveland mapping (Fig. 1*c*) confirms that the phosphoprotein in untreated *ras*-transformed cells is the same protein that is phosphorylated in response to PMA. A correlation plot of basal phosphorylation level versus DG concentration for the cell lines tested gave a regression coefficient of 0.993,

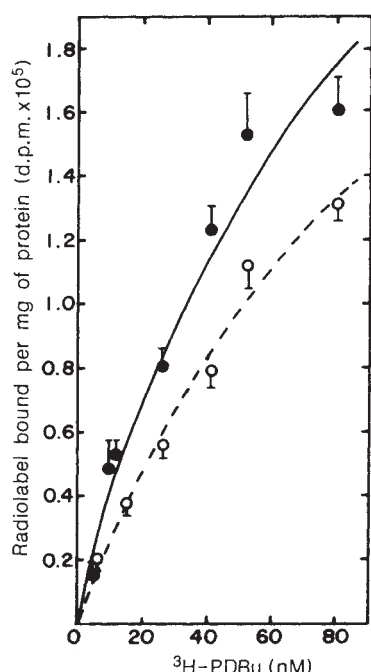


Fig. 2 Phorbol ester binding to intact NIH 3T3 and Harvey-transformed 3T3 cultures. Binding to wild-type NIH 3T3 (●) and Ha 1-3T3 (○) shown. Exponentially growing cells in 35-mm dishes were washed once with 5 ml PBS and once with binding buffer (2 vol. DMEM, 1 vol. PBS, 1 mg ml⁻¹ BSA)²⁰. The cells were then incubated for 1 h with binding buffer before addition of phorbol esters. ³H-PDBu (NEN, 15.8 Ci mmol⁻¹) was added to the cultures in dimethylsulphoxide. Nonspecific binding was assessed by addition of a 200-fold excess of PMA. After 30 min at 37 °C, the medium was aspirated off, the dishes washed through 250 ml binding buffer containing 0.1 mg ml⁻¹ fatty-acid-free BSA, then 4 × 250 ml PBS (4 °C). Residual liquid was aspirated off and 700 µl of 0.1 M NaOH was added. After 1 h at 37 °C, the solution was transferred to a microfuge tube and the dishes washed with another 700 µl 0.1 M NaOH. A sample of the combined solution (1 ml) was taken for determination of ³H and 200 µl was used for protein determination via the Bradford assay²¹. Data were normalized to d.p.m. bound per mg of protein. The data were fit by a nonlinear least-squares program with a binding equation describing a single independent class of sites. The estimated K_D for the NIH 3T3 cells was 80 nM with a total of 6.0×10^{12} binding sites per mg protein. The Ha 1-3T3 cells had a K_D of 84 nM with a total of 4.5×10^{12} binding sites per mg of protein.

indicating a very close correlation between these two parameters. The results suggest that the constitutively elevated DG levels in the transformed cells are sufficient to partially activate protein kinase C while also down-regulating the response to PMA.

To test the possibility that the apparent down-regulation was a consequence of a very slow turnover of phosphate on the 80K protein, we labelled cells for either 1 or 6 h with ³²P-phosphate before challenging with phorbol ester. But the blunted response of the transformed cells to phorbol ester was independent of the incubation period (data not shown). Other explanations include down-regulation of the 80K substrate itself, or down-regulation of protein kinase C. The latter effect has recently been documented in response to chronic administration of phorbol esters¹⁵, but has never been reported as an effect of changes in endogenous levels of DG. We therefore measured phorbol ester binding to intact control and Ha-*ras*-transformed cells using ³H-PDBu (Fig. 2). Although the apparent dissociation constants for the two cell lines were about the same (80 nM), maximal specific binding to the transformed cells (Ha-1) was ~25% lower than that to control cells, suggesting that the decreased responsiveness to phorbol ester can at least in part be explained by a decrease in available protein kinase C.

Table 3 PMA-induced phosphorylation of the 80K protein

Cell type	PMA	% 80K phosphorylation	Fold stimulation
3T3	—	2.8 ± 0.64	
3T3	+	100.0	35.7
Ha 1-3T3	—	10.5 ± 2.8 (n = 4)	
Ha 1-3T3	+	39.3 ± 7.4 (n = 4)	3.74
Ha 2-3T3	—	14.3 (n = 2)	
Ha 2-3T3	+	45.5 (n = 2)	3.18
KSV-3T3	—	53.3 (n = 2)	
KSV-3T3	+	90.3 (n = 2)	1.69

Samples were prepared and analysed by SDS-polyacrylamide gel electrophoresis as in Fig. 1a. Quantitation was achieved by using pre-flashed Kodak XAR film and scanning the resulting autoradiographs on an LKB Laser Ultrascanner. The peaks corresponding to the 80K protein were cut out with a razor blade and weighed on a microgram balance.

The data presented are consistent with the hypothesis that transformation by *ras* oncogenes results in a constitutive partial activation and down-regulation of protein kinase C, by elevation of cellular DG. These effects are not merely a reflection of differences in proliferative state that occur at confluence because all experiments were performed using sparse cultures in exponential growth, nor are they artefactual differences caused by long-term cell-line divergence, by viral infection, or by selection of idiosyncratic clones. It is not yet known whether the increase in DG is responsible for the phenotypic transformation associated with activated *ras* genes, although prolonged (1 day) incubation of NIH 3T3 cells with PMA (10 nM) induces a morphological state similar to that of *ras*-transformed 3T3 cells (data not shown). The mechanism by which DG is elevated in the transformed cells is also unclear, particularly as the steady-state levels of all other PI metabolites were unchanged and addition of lithium did not cause the accumulation of InsP. However, nonequilibrium ³²P-labelling experiments have revealed small but significant differences in the rates of phosphoinositide phosphorylations between control and transformed cells (A.W. and I.G.M., manuscript in preparation), suggesting that a small, labile pool of phosphatidylinositol may be regulated, directly or indirectly, by *ras* oncogene products.

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Modulation of gelsolin function by phosphatidylinositol 4,5-bisphosphate

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The actin-binding protein gelsolin requires micromolar concentrations of calcium ions to sever actin filaments, to potentiate its binding to the end of the filament and to promote the polymerization of monomeric actin into filaments¹⁻⁵. Because transient increases in both intracellular $[Ca^{2+}]^{6-8}$ and actin polymerization^{8,9} accompany the cellular response to certain stimuli, it has been suggested that gelsolin regulates the reversible assembly of actin filaments that accompanies such cellular activations. But other evidence¹⁰⁻¹² suggests that these activities do not need increased cytoplasmic $[Ca^{2+}]$ and that once actin-gelsolin complexes form in the presence of Ca^{2+} *in vitro*, removal of free Ca^{2+} causes dissociation of only one of two bound actin monomers from gelsolin and the resultant binary complexes cannot sever actin filaments^{4,13-15}. The finding that cellular gelsolin-actin complexes can be dissociated¹⁶ suggests that a Ca^{2+} -independent regulation of gelsolin also occurs. Here we show that, like the dissociation of profilin-actin complexes¹⁷, phosphatidylinositol 4,5-bisphosphate, which undergoes rapid turnover during cell stimulation^{18,19}, strongly inhibits the actin filament-severing properties of gelsolin, inhibits less strongly the nucleating ability of this protein and restores the potential for filament-severing activity to gelsolin-actin complexes.

Actin filaments (F-actin) exist in a steady state with actin monomers (G-actin) (see ref. 20 for review). Dilution of F-actin causes depolymerization to maintain a critical concentration of G-actin. The rate of depolymerization is proportional to the number of unbound, slowly exchanging ends of actin filaments²¹⁻²³ and is also proportional to the number of cuts made by the severing protein gelsolin. The rate of depolymerization can be measured by pyrene-iodoacetamide labelling²⁴. The severing function of gelsolin can be almost completely eliminated by low concentrations of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂), even in the presence of 200–1,000 μM Ca^{2+} . The site affected by PtdIns(4,5)P₂ seems to be in the N-terminal half of gelsolin as the severing function of this part of the molecule²⁵⁻²⁷, which is not calcium-sensitive, is also inhibited by PtdIns(4,5)P₂ (Fig. 1b). The effect of the lipid is highly specific, as there is no inhibition by phosphatidylinositol (PtdIns), phosphatidic acid, 1-oleoyl-2-acetylgllycerol (OAG), inositol triphosphate (InsP₃), glycerophosphoinositol 4,5-bisphosphate or phosphatidyl serine. Phosphatidylinositol monophosphate (PtdIns(4)P) exhibited a slight (<10 per cent) inhibitory effect, suggesting that it also inhibits gelsolin, but the possibility of minor contamination with PtdIns(4,5)P₂ was not excluded. Treatment of PtdIns(4,5)P₂ with phospholipases A₂ or C or with an excess of the non-ionic detergents Triton X-100 or Tween-20 before addition to gelsolin eliminates its inhibitory effect. In contrast to the effect on severing, the ability of gelsolin to nucleate filament assembly is much less inhibited by an equivalent amount of PtdIns(4,5)P₂ (Fig. 1c).

Half-maximal inhibition of the severing function of 86 nM gelsolin is observed with 2 μg ml⁻¹ (1.7 μM) PtdIns(4,5)P₂ (Fig. 2). Aqueous dispersions of PtdIns(3,4)P₂ consist of small micelles of relative molecular mass 82–93,000 rather than the large bilayer vesicles characteristically formed by other phospholipids with two acyl chains²⁸. The physical state of PtdIns(4,5)P₂ under the experimental conditions used here was examined by quasielastic light scattering. The scattering intensity of this phospholipid in the concentration range 40–120 μg ml⁻¹ is three orders of magnitude less than that of equivalent concentrations

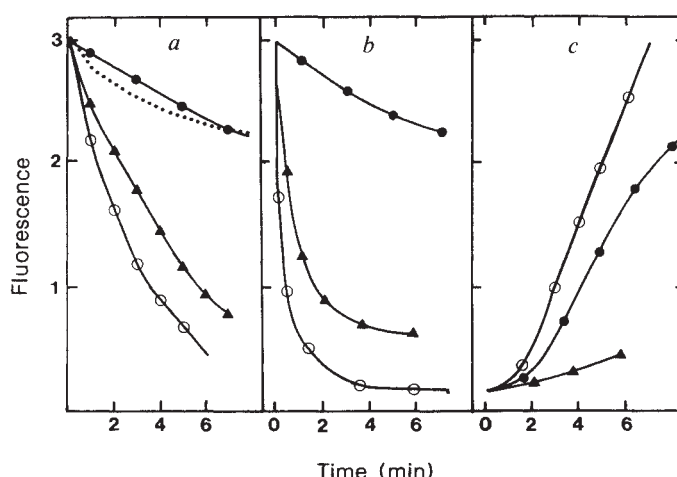


Fig. 1 Effects of phosphoinositides on severing or nucleation of actin filaments by gelsolin. *a*, Fluorescence of filamentous actin. Rates of fluorescence decrease, proportional to the rate of actin depolymerization in the absence (○) or presence of PtdIns(4,5)P₂ (●) or PtdIns(4)P (▲) are shown. Fluorescence is reported in arbitrary units with that of filamentous actin equal to 3 and monomeric actin equal to 0.12. Only PtdIns(4,5)P₂ and PtdIns(4)P caused a significant change in the depolymerization rate (negative data omitted for clarity). The fluorescence of F-actin diluted in the absence of gelsolin is shown by the dotted line. *b*, Experiment similar to that described in *a* was performed using 40 nM of the thermolysin-derived N-terminal fragment of gelsolin²⁷. The effects of 40 μg ml⁻¹ PtdIns(4)P (▲) and 40 μg ml⁻¹ PtdIns(4,5)P₂ (●) are compared with the rate in the absence of added phospholipid (○). *c*, The polymerization of pyrene-labelled actin (4 μM) was accelerated by addition of solution B containing 45 nM gelsolin without (○) or with (●) 40 μg ml⁻¹ PtdIns(4,5)P₂ compared with the rate in the absence of gelsolin (▲). An equal amount of PtdIns(4)P caused no decrease in the actin polymerization rate under these conditions. The fluorescence of F-actin on this arbitrary scale was 7.

Methods. Macrophage gelsolin purified according to Chaponnier *et al.*²⁷ was made 25 nM in a solution containing 2 mM Tris, 0.2 mM CaCl₂, 0.2 mM ATP, 0.2 mM 2-mercaptoethanol, 2 mM MgCl₂ and 150 mM KCl (solution B). To 390 μl of the gelsolin solutions were added 10 μl of solution B or 1 mg ml⁻¹ PtdIns(4,5)P₂, PtdIns(4)P, PtdIns, phosphatidic acid, phosphatidyl serine or InsP₃ (Sigma), lipids dispersed in water with 15 s sonication at maximum setting in an Ultrasonics cell disrupter with the exception of OAG which was dissolved at a concentration of 1 mg ml⁻¹ in dimethylsulphoxide. After 1 min, 5 μM polymerized actin purified³⁶ and pyrene-labelled²⁴ was added to give a final concentration of 200 nM (below the critical concentration of the slow-exchanging ends of actin filaments (0.5–1.0 μM) under these conditions), and the fluorescence at 386 nm with excitation at 365 nm was measured in a Perkin-Elmer LS-5 instrument with 3-nm slit width.

of PtdIns. PtdIns exhibits a diffusion constant of 1.1×10^{-8} cm² s⁻¹, whereas the diffusion constant of PtdIns(4,5)P₂ was too fast to measure, confirming that large vesicles were not present. Because PtdIns(4,5)P₂ is presumably in the form of micelles of packing number 82 (ref. 28), half the gelsolin (42 nM) is inactivated by 20 nM of these micelles, suggesting that the interaction between gelsolin and PtdIns(4,5)P₂ is of high affinity. But the nucleating activity of gelsolin is only slightly inhibited by these concentrations.

The inhibitory effect of PtdIns(4,5)P₂ is reversed by incubation with Triton X-100 before addition to gelsolin (Fig. 2, inset). The restoration of activity depends strongly on the order in which Triton, PtdIns(4,5)P₂ and gelsolin are added. Incubation of 22 μM PtdIns(4,5)P₂ with 3,900 μM (0.25%) Triton X-100 for 30 s before addition to 86 nM gelsolin preserves 97% of the severing activity, but incubation of PtdIns(4,5)P₂ with gelsolin for 30 s before addition of Triton restores only 60% of the

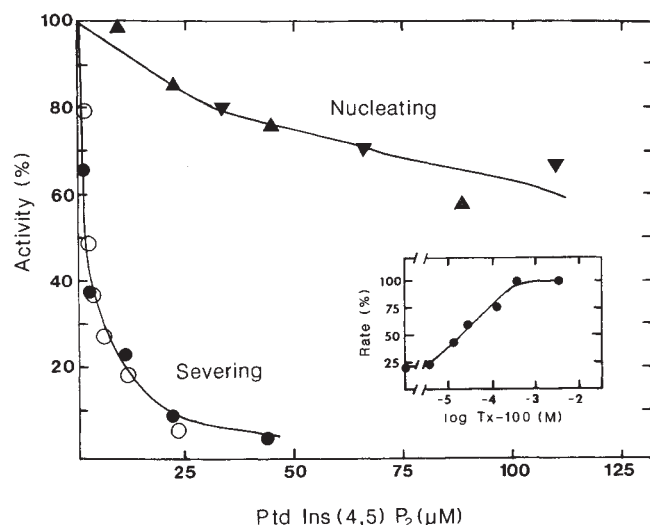


Fig. 2 Concentration dependence of the inhibition of severing and nucleating activity by PtdIns(4,5)P₂ and the reversal of inhibition by detergent. Actin-filament-severing activity of gelsolin was measured by adding pyrene-labelled F-actin (final concentration 200 nM) to 86 nM gelsolin and various amounts of PtdIns(4,5)P₂ in solution B. The relative severing activity (●) was assumed to be proportional to the initial rate of fluorescence decrease during the depolymerization of the severed filaments. To confirm that the pH of the solutions was maintained by buffer B, one set of measurements used solution B supplemented by 20 mM Tris (pH 7.6) (open circles). Upper curve, relative nucleating activity measured from the initial rate of fluorescence increase observed following addition of 3.3 μM pyrene-labelled G-actin to gelsolin and different concentrations of PtdIns(4,5)P₂ in solution B. ▲, ▼ separate experiments using 60 or 86 nM gelsolin, respectively. Inset, relative rates of actin depolymerization following dilution of pyrene-labelled F-actin to 200 μM in solution B with 16 nM gelsolin, 18 μM PtdIns(4,5)P₂ and Triton X-100.

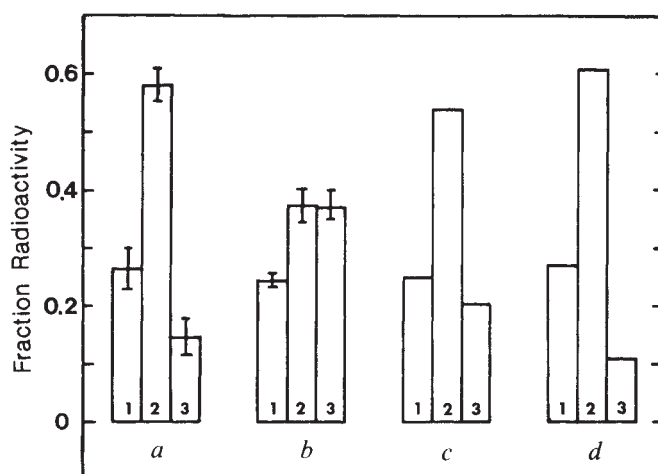


Fig. 3 Binding of radiolabelled PtdIns(4,5)P₂ to gelsolin. [³H]PtdIns(4,5)P₂ in methylene chloride: ethanol: water (20:10:1) (2.0 Ci mmol⁻¹, NEN) was dried under vacuum, dispersed at a concentration of 1 mg ml⁻¹ in water with sonication for 15 s diluted to 38 μg ml⁻¹ in a solution containing 50 mM Tris, 150 mM NaCl, pH 7.4 (TBS) and 400 μl mixed with 30 μl 4 μM macrophage gelsolin and 20 μl of a Sepharose bead suspension to which monoclonal anti-gelsolin IgG was covalently coupled (b). Phospholipase A₂ (c) or phospholipase C (d) (Sigma) was added to some samples. Control samples were made by addition of [³H]PtdIns(4,5)P₂ alone to anti-gelsolin beads without added gelsolin (a). Solutions were incubated with anti-gelsolin beads for 1 h at room temperature with end-over-end rotation. The beads were then sedimented by centrifugation at 18,000g for 1.5 min. Of the supernatant solution, 400 μl was removed, and 400 μl TBS containing 1% v/v Triton X-100 was added with gentle mixing and the beads were sedimented within 30 s after adding Triton, as described above. Supernatant solution, 400 μl was again removed and the residual pelleted material was suspended in a total volume of 400 μl TBS. The radioactivity of the TBS supernatant solution (1), the TBS-Triton supernatant solution (2) and the suspended Sepharose beads (3) were determined by adding 50 μl of each sample to 10 ml of scintillation fluid (AquaSol, DuPont) and counting in a Packard liquid scintillation spectrometer. Error bars, standard deviation from 4 (a) or 3 (b) separate experiments.

severing function. When gelsolin-PtdIns(4,5)P₂ was added to 200 nM F-actin followed by Triton, only 20–30% of the severing function was restored. Very similar results were observed using Tween 20, another non-ionic detergent. These results suggest that the effect of detergent is not simply to replace PtdIns(4,5)P₂ in equilibrium binding, but may involve a disruption of the micelle structure.

When gelsolin was incubated with [³H]PtdIns(4,5)P₂ and subsequently immunoprecipitated with monoclonal anti-gelsolin immunoglobulin G (IgG) coupled to Sepharose beads, 37 ± 3% of the radioactivity remains bound to the beads after brief successive washes with buffer and 1% Triton X-100 (Fig. 3b). In control experiments without gelsolin in the incubation mixture, 15 ± 3.5% of radioactivity remains bound (Fig. 3a). Addition of phospholipase A₂ or C to gelsolin-PtdIns(4,5)P₂ lowers the amount of bound radioactivity to control levels. Similar experiments using [³H]InsP₃ demonstrate no binding of InsP₃ to gelsolin.

As well as its effects described above, PtdIns(4,5)P₂ is the first non-denaturing agent discovered that restores the severing function of gelsolin after the protein has formed complexes with actin in Ca²⁺ (Fig. 4A). When unlabelled actin-gelsolin complexes are added to pyrene-labelled F-actin, and the mixture is diluted to below the critical concentration of the slow-exchanging ends, the rate of depolymerization of the labelled actin filaments is relatively slow (▲), because actin-gelsolin complexes block the fast-exchanging filament ends but do not increase the number of slowly exchanging ends by severing filaments. Treatment of the complexes with EGTA does not restore their ability to sever filaments⁴, but when they are reacted with PtdIns(4,5)P₂, added to labelled filaments and the inhibi-

tory effect partially reversed by addition of 0.25% Triton X-100, the labelled filaments depolymerize at an accelerated rate (●), almost the same as that observed in the absence of prior formation of unlabelled complexes (○). Incomplete restoration of severing function is consistent with the order in which gelsolin, PtdIns(4,5)P₂ and Triton were added.

The amounts of actin that co-sediment with gelsolin after 2:1 actin-gelsolin complexes are exposed to different factors are shown in Fig. 4B. Treatment of the complexes with PtdIns(4,5)P₂ in the presence of Ca²⁺ reduces the molar ratio of actin bound to gelsolin from 2.14 ± 0.11 (a) to 1.26 ± 0.22 (b), a decrease comparable with that caused by an excess of EGTA which diminishes the molar ratio to 1.12 ± 0.24 (c). Exposure of the 2:1 actin-gelsolin complex sequentially with PtdIns(4,5)P₂ followed by EGTA decreases the molar ratio to 0.72 ± 0.09 (d). In other experiments (not shown), incubation of the complexes with PtdIns(4,5)P₂ and vitamin D-binding protein (which removes actin from the EGTA-sensitive actin-binding site on gelsolin²⁹) also reduces the actin:gelsolin ratio to ~0.7. Thus, PtdIns(4,5)P₂ can also remove one of the actin monomers from the 2:1 actin-gelsolin complex in the presence of Ca²⁺. As this lipid renders gelsolin-actin complexes capable of severing actin filaments whereas EGTA and vitamin D-binding protein do not^{4,29}, we conclude that PtdIns(4,5)P₂ promotes the dissociation of a different actin molecule than does EGTA or the vitamin D-binding protein, which act on the calcium-sensitive actin

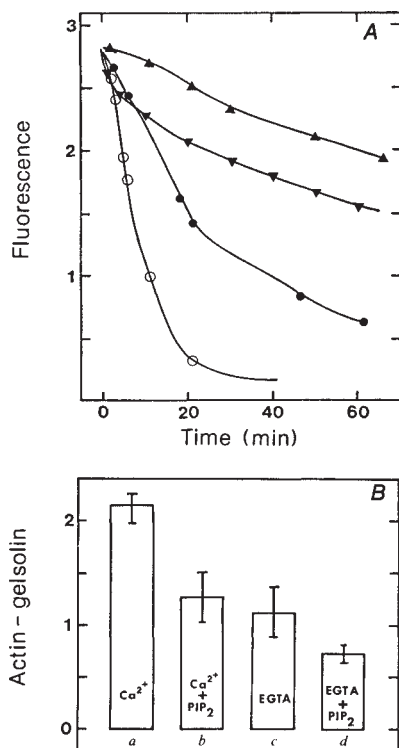


Fig. 4 *A*, Restoration of the severing activity of Gelsolin-actin complexes by PtdIns(4,5)P₂; *B*, effects of PtdIns(4,5)P₂ on the dissociation of actin from gelsolin. *A*, Gelsolin (1 μ M) was mixed with unlabelled G-actin (2 μ M) in solution A. After overnight incubation at 4 °C the gelsolin-actin mixtures were diluted 40-fold in solution B (▲) or solution B with 25 μ g ml⁻¹ PtdIns(4,5)P₂ (●). Pyrene-labelled F-actin was then added to give a concentration of 200 μ M (below critical concentration) and after 1 min, Triton X-100 was added to give a final concentration of 0.025% to reverse partially the inhibitory effect of PtdIns(4,5)P₂. The rate of depolymerization of the labelled actin filaments was then measured by the decrease of fluorescence following addition of Triton. Less than 5% of the fluorescence decrease occurred before addition of Triton. The rates of depolymerization are compared with those of F-actin diluted in the same manner as above into solution B with 25 μ g ml⁻¹ PtdIns(4,5)P₂ in the absence of gelsolin (▼) or into buffer B with PtdIns(4,5)P₂ and gelsolin that had not been incubated with unlabelled actin (○). In the latter two experiments, Triton X-100 was also added. *B*, Gelsolin was mixed with G-actin at a 2:1 molar ratio in solution B from which MgCl₂ and KCl were omitted (solution A) to prevent actin polymerization. To identical samples (3 μ M gelsolin in 50 μ l) were added 350 μ l diluent alone (*a*), 50 μ g ml⁻¹ PtdIns(4,5)P₂ in diluent (*b*), 1 mM EGTA (*c*) or PtdIns(4,5)P₂ and EGTA (*d*). After 5 min at room temperature, 20 μ l anti-gelsolin-Sepharose beads were added and rotated for 30 min, and the beads sedimented by centrifugation as described in Fig. 3 legend. The beads were washed once with 500 μ l solution A and sedimented. After removal of the supernatant, polypeptides associated with the beads were resolved by SDS-PAGE. The relative amounts of proteins were determined by quantitative densitometry of Coomassie blue-stained bands. Error bars, results of 4 separate experiments; 3 using macrophage gelsolin (2 different preparations) and 1 using plasma gelsolin.

complexes from rabbit macrophages show very similar results (K. Iida, unpublished experiments).

The effects of PtdIns(4,5)P₂ on the function of gelsolin were examined using both cytoplasmic gelsolin from rabbit macrophages and human plasma gelsolin. We found no functional differences between the two types, suggesting that the effect of PtdIns(4,5)P₂ does not depend on the N-terminal extension present in the plasma form³⁰. The reversibility of the effects of PtdIns(4,5)P₂ by hydrolysis with phospholipase A₂ or C suggests that the acyl chains of PtdIns(4,5)P₂ may need to form contacts with a hydrophobic domain of gelsolin. This postulated highly hydrophobic surface domain of gelsolin is also consistent with solvent effects on its intrinsic fluorescence³¹ and its high affinity for phenyl-Sepharose³². In addition, the amino-acid sequence of gelsolin³⁰ contains one region, in the N-terminal half, identical to a sequence thought to be responsible for phospholipid binding to other Ca²⁺-binding proteins³³ (D. J. Kwiatkowski, personal communication).

Although we do not know how PtdIns(4,5)P₂ interacts with profilin¹⁷ or with gelsolin (this paper) *in vivo*, our findings seem to relate the well-documented turnover of PtdIns(4,5)P₂ during cell activation to the assembly of actin filaments^{8,9,18,19,34,35}, and suggest that PtdIns(4,5)P₂ is the second messenger, in a similar manner to InsP₃ in other systems. The amount of PtdIns(4,5)P₂ turnover *in vivo*^{3,4} is sufficient to alter the activity of both gelsolin and profilin, especially if there is preferential co-localization of these cell components. A coupling of the dissociation of profilin-actin and gelsolin-actin complexes by PtdIns(4,5)P₂ would promote the nucleated assembly of actin. According to this scheme, an initial calcium-dependent nucleation and severing of actin filaments by gelsolin, following perturbation of appropriate cell receptors, would increase the number of nuclei on which actin filaments could grow.

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binding domain in the C-terminal half of gelsolin^{25,27}. On the other hand, although sequential treatment of gelsolin-actin complexes with PtdIns(4,5)P₂ and EGTA further reduces the proportion of actin tightly bound to gelsolin, the former does not remove all actins associated with gelsolin. This result agrees with our data showing that PtdIns(4,5)P₂ does not totally restore the severing ability of gelsolin-actin complexes. In these experiments we used macrophage gelsolin bound to muscle actin. Additional experiments with EGTA-resistant gelsolin-actin

Glucocorticoid receptor mutants that are constitutive activators of transcriptional enhancement

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Glucocorticoids, a class of steroid hormones, associate specifically with intracellular receptors, facilitating a conformational change that converts the receptor *in vitro* to a DNA-binding protein¹ and *in vivo* to a nuclear species² that activates a class of transcriptional enhancers termed glucocorticoid response elements^{3,4} (GREs). The DNA sequences recognized specifically by the hormone-receptor complex correspond directly to those required for GRE enhancement^{4,5}. The structural transition that accompanies steroid binding, 'receptor transformation', has been monitored by changes in receptor chromatographic properties⁶, accessibility to monoclonal antibodies⁷, association with other receptor subunits or with heterologous proteins^{8,9}, and aqueous two-phase partition coefficient^{10,11}. However, the significance of the structural change for the biological activity of the receptor is not understood. We have used cloned rat glucocorticoid-receptor coding sequences¹² to produce and characterize a novel class of receptor mutants that elicit GRE enhancer function in transfected cells even in the absence of hormone. The constitutive activity of those receptor derivatives, together with mapping studies that distinguish between the DNA- and hormone-binding domains of the receptor, imply that the conformational change corresponding to receptor transformation may simply unmask pre-existing functional domains for DNA binding, enhancer activation, or both.

Genetic¹³ and molecular^{14,15} studies have implied that the glucocorticoid receptor carries determinants for DNA binding and hormone binding in its carboxy-terminal half. To localize these regions more directly, complementary DNA fragments encoding the entire 795-amino-acid rat glucocorticoid receptor¹², or specific subregions of it (Fig. 1a), were transcribed *in vitro* using SP6 RNA polymerase, and the mRNAs produced were translated in reticulocyte lysates. The receptor derivatives were incubated with a mixture of six end-labelled DNA fragments; one contains a functional GRE with five consensus binding sites for receptor, two contain a single consensus sequence each, and three lack specific binding sequences (Fig. 1b). Bound fragments were isolated by immunoprecipitation with receptor-specific monoclonal antibodies^{16,17}, released from the complexes, and separated on agarose gels. Figure 1b shows that the affinity and selectivity of the intact receptor synthesized *in vitro* were apparently indistinguishable from the DNA-binding activity of receptor purified from rat liver. In fact, specific DNA-binding activity was retained by a receptor fragment containing amino acids 407-566; in contrast, a fragment containing amino acids 407-492 failed to bind DNA (Fig. 1a, b), as did other receptor derivatives with deletions between amino acids 450 and 495 (data not shown).

Parallel studies were carried out to localize the hormone-binding region (Fig. 1a); binding of ³H-dexamethasone was assessed using a filter-binding assay¹². We found that a receptor derivative containing residues 407-795 bound hormone indistinguishably from intact receptor, whereas deletion of 29 carboxy-terminal amino acids (receptor fragment 407-766) reduced hormone binding by 100-fold, and a receptor fragment containing amino acids 547-795 displayed a 10-fold reduction in binding; further amino-terminal deletions resulted in progressively greater losses in activity (Fig. 1a). Although these experiments do not position precisely the DNA- and hormone-binding

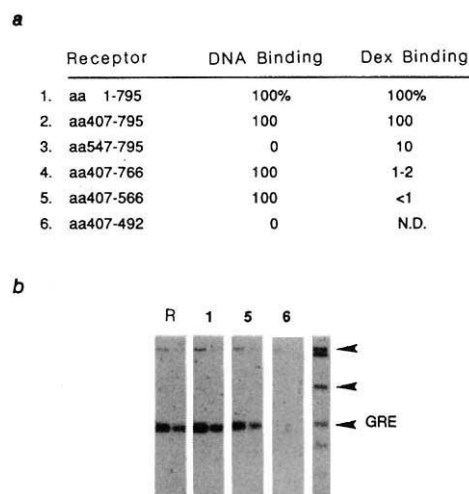


Fig. 1 Localization of hormone- and DNA-binding domains. Receptor cDNA fragments encompassing the complete open reading frame (amino acids 1-795) or the subregions indicated were cloned into pSP64 and transcribed *in vitro* using SP6 RNA polymerase; the resultant mRNAs were then translated in reticulocyte lysates. Methods were as described¹². Fragments encoding receptor subregions were generated by cleavage at convenient restriction sites, usually followed by resection with exonuclease *Bal31*. After insertion into appropriate expression vectors, all constructions were characterized by DNA sequencing. Amino-terminal deletion derivatives were fused to sequences encoding the first three amino acids of herpes simplex virus thymidine kinase; translation of carboxy-terminal deletion derivatives extended to termination codons within pSP64 sequences, resulting in the addition of 6-34 nonreceptor amino acids. A portion of each translation reaction was labelled with ³⁵S-methionine to confirm the predicted sizes of the translation products and to normalize protein levels for the binding reactions. **a**, DNA binding represents relative selective binding to a specific restriction fragment containing a GRE element, as measured in an immunoprecipitation assay (see below). To measure hormone (Dex.) binding, translation reactions (20 °C, 30 min) were carried out in 0.1 μM ³H-dexamethasone, and in the presence or absence of a 200-fold excess of unlabelled dexamethasone; products were then assayed for hormone binding using a filter-binding method¹²; specific binding was calculated as the difference in bound radioactivity in the presence and absence of unlabelled dexamethasone. **b**, Specific DNA binding by (lane R) the glucocorticoid receptor protein purified from rat liver²⁴ and (lanes 1, 5, 6) by *in vitro* translation products (see **a**). Intact and mutant receptors (0.1 pmol) were immunoprecipitated with monoclonal antibodies^{16,17}, and incubated³⁵ with 20 ng end-labelled *Xho*II fragments (right-hand lane) of a plasmid containing the MTV LTR GRE within the indicated fragment, and single consensus octamers for receptor binding within the two fragments marked with arrows. Bound DNA fragments were eluted and separated in agarose gels. The paired lanes represent assays containing 20 ng and 40 ng (left and right, respectively) of pBR322 nonspecific competitor DNA.

domains (detailed mapping studies will be presented elsewhere; S.R. and K.R.Y., in preparation), they demonstrate that the two binding activities reside in distinct regions of the carboxy-terminal half of the receptor, although sequences near the DNA-binding domain clearly influence hormone binding.

We propose two general mechanisms by which hormone binding might yield the functional enhancer-activating protein. The ligand-receptor interaction might trigger a conformational change in the protein which results in the formation of the DNA-binding or enhancer-activation domains (Fig. 2a). Alternatively, the ligand-stimulated structural transition might unmask (derepress) DNA-binding or enhancer-activation domains already folded in their functional configurations (Fig. 2b). The 'induction model' predicts that mutant receptors

a Induction



b Derepression



Fig. 2 Diagrammatic view of two mechanisms by which the structural change in the receptor might yield the biologically active species. Square forms, unoccupied receptors; triangular notch, hormone-binding site. Receptor transformation, which occurs upon hormone binding at moderate temperatures, yields the functional receptor species, represented as the circular form; broken line, the DNA-binding domain; square notch, putative enhancer-activation domain. DEX, dexamethasone. *a*, Induction model: hormone binding induces formation of the DNA-binding/enhancer-activation domains. *b*, Depression model: hormone binding relieves inhibition of the DNA-binding/enhancer-activation domains.

lacking the hormone-binding domain would fail to activate GRE enhancers, whereas the 'derepression model' predicts that constitutive enhancer activating proteins, functional even in the absence of hormone might appear upon deletion of a region that masks or represses otherwise functional DNA-binding or enhancer-activation domains.

Receptor cDNA fragments encoding proteins bearing nested carboxy-terminal deletions (Fig. 3*a*) were inserted into the simian virus (SV40)-based expression vector pSV7d (ref. 12), and GRE enhancer activation activity was tested in transient cotransfections with a reporter plasmid carrying a GRE-linked chloramphenicol acetyltransferase (CAT) gene into CV-1 cells, which contain little or no active receptor. As expected, cultures cotransfected with the intact receptor coding sequence and incubated with 0.1 μ M dexamethasone produced >100-fold higher levels of CAT activity than parallel cultures not treated with hormone (Fig. 3*b*). Four mutant receptors, lacking 27, 101, 123 and 180 carboxy-terminal amino acids (Fig. 3*b*), failed to activate GRE enhancer function either in the presence or in the absence of dexamethasone. Similarly, deletion mutants encoding receptor fragments 1-508 and 1-464, which lack the carboxy-terminal 287 and 331 amino acids, respectively, yielded no GRE activation. In striking contrast, receptor derivatives with deletions of 190, 200, 239 and 270 carboxy-terminal amino acids displayed constitutive enhancer activation, producing elevated CAT activities even in the absence of added hormone. Similar results (not shown) were obtained when these same receptor derivatives were tested in the receptor-deficient HTC cell mutant line 6.10.2 (ref. 12), and in COS-7 cells¹⁸.

Results of the CAT assays were confirmed and extended by direct quantification of transcripts initiated at the regulated promoter. In these experiments, COS-7 cells were cotransfected with a receptor plasmid, the reporter plasmid pLT1 (ref. 19), and an internal control plasmid pRSVCAT (ref. 20). RNA from the transfected cells was then assayed by primer extension using oligonucleotides complementary to the mouse mammary tumour virus (MTV) LTR and CAT transcripts; these primers measure initiation from the GRE-linked MTV promoter of pLT1, and from the Rous sarcoma virus promoter of pRSVCAT, respectively. For example, Fig. 3*c* shows that mutant receptor 1-556, which lacks 239 carboxy-terminal amino acids, strongly stimulates initiation from the GRE-linked promoter both in the presence and absence of hormone. Thus, the amino-terminal 556 amino acids of receptor activate GRE enhancer function constitutively, yielding expression comparable to that induced by the intact receptor-hormone complex.

To examine directly the receptor species produced in these experiments, parallel transfected cultures were metabolically labelled with ³⁵S-methionine and ³⁵S-cysteine for 16 h beginning 40 h after transfection; labelled receptors were immunoprecipitated and examined after SDS-polyacrylamide gel electrophoresis. A portion of those results is shown in Fig. 4, revealing approximately equivalent labelling intensities for the full-length receptor, constitutive mutant 1-556 and nonfunctional derivative 1-768. Thus, we conclude that the defects in the nonfunc-

tional species do not merely reflect failures of synthesis or accumulation, and that the specific activity of constitutive mutant 1-556 is similar to that of the hormone-associated intact receptor.

The experiments presented here confirm and extend our earlier conclusions that the DNA- and hormone-binding domains of the glucocorticoid receptor reside in the carboxy-terminal half of the protein^{14,15}, and that the DNA-binding region is positioned near the middle of the coding sequence¹⁵. Recent studies of the human glucocorticoid and oestrogen receptors identify segments of those proteins apparently responsible for nuclear localization²¹ and ligand binding^{21,22}; these correspond closely to the respective regions of the rat glucocorticoid receptor that we ascribe to DNA- and dexamethasone-binding. Surprisingly, even small fragments encompassing the DNA-binding domain appear to retain DNA-binding affinity and selectivity similar to the intact receptor. In contrast, Ogata and Gilbert²³ showed that the isolated DNA-binding domain of the *lac* repressor could selectively recognize the *lac* operator sequence, but bound with ~100-fold reduced affinity. Thus, the receptor protein sequences involved in specific DNA binding appear to fold into a highly stable structure, and to exhibit the properties of a functional domain.

The position of the hormone-binding domain has been inferred predominantly from loss-of-function mutations, some of which may reflect lesions that alter receptor folding but do not involve sequences in the hormone-binding site itself. This is consistent with previous reports that hormone bound to the intact receptor remains associated with specific fragments after proteolysis, whereas similar fragments generated in the absence of hormone subsequently fail to bind ligand²⁴⁻²⁶. The present results, together with the receptor proteolysis studies, ligand-binding site affinity mapping (S. Simons, personal communication), and additional deletion-mutant analyses (S.R. and K.R.Y., in preparation), support the view that the hormone-binding domain is near the receptor carboxy terminus, and that the hormone-binding site might be a relatively unstable structure in the absence of sequences extending towards the DNA-binding domain.

The simplest interpretation of our results is that hormone-mediated receptor activation corresponds to a conformational derepression of the preformed DNA-binding domain, enhancer activation domain, or both. Although other models cannot unequivocally be excluded without detailed molecular structure analyses, several aspects of our findings support our interpretation. First, four extensive deletion mutants that retain the intact DNA-binding domain give rise to constitutive activators, whereas four mutants lacking fewer carboxy-terminal amino acids are nonfunctional. Second, the DNA-binding domain appears to be structurally autonomous. Finally, numerous drastic deletions within the amino-terminal half of the receptor retain hormone-dependent enhancement activity²⁷, implying that the structure of the functional domains is independent of other regions of the receptor. Conceivably, then, the portion of the receptor that is deleted may normally mask the functional

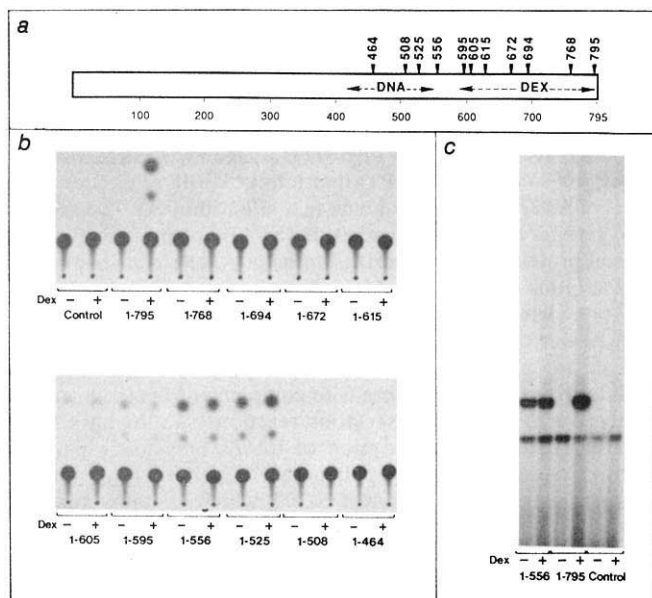


Fig. 3 Effect of carboxy-terminal receptor deletions on GRE enhancer activation. *a*, Diagram of the rat glucocorticoid receptor showing the regions involved in DNA and hormone binding, and the carboxy-terminal deletion mutants used for the DNA transfection studies. The precise borders of the DNA- and hormone-binding domains are not defined in this study, and are therefore represented as dotted lines. Translation of the deletion derivatives terminates at one of three stop codons within the polylinker into which the cDNA was inserted; each therefore contains additional amino acids (4–16) encoded by the polylinker. Each recombinant is marked with the receptor amino acids included in the construct; the complete open reading frame is 1–795. *b*, Parallel cultures of CV-1 cells (10^6) were cotransfected³⁶ with 5 μ g of a plasmid encoding the indicated receptor derivative together with 1 μ g of the reporter plasmid pGMCS (ref. 36), which carries a GRE-linked *cat* gene driven by the MTV promoter; DNA titration studies (not shown) confirmed that these plasmid levels produced CAT activities within the linear range under our assay conditions. After 4 h, the DNA precipitates were replaced with Dulbecco's modified Eagle's medium (supplemented with 10% fetal calf serum) either containing (+) or lacking (–) 0.1 μ M dexamethasone; extracts were prepared after an additional 36 h at 37 °C. The autoradiogram shows a standard thin-layer chromatographic assay of CAT activity³⁷ using 14 C-chloramphenicol as substrate. *c*, Primer extension analysis of reporter-gene transcripts produced in receptor cotransfections. Parallel cultures of COS-7 cells (3×10^6) were cotransfected with the indicated receptor plasmid (15 μ g), the reporter plasmid pLTL1 (15 μ g; ref. 19), and the internal control plasmid pRSVCAT (1.5 μ g; ref. 20). After subsequent propagation in the presence or absence of dexamethasone, total RNA was prepared; 75 μ g RNA was hybridized with 32 P end-labelled primers complementary to LTL1 transcribed sequences +50 to +79 and RSVCAT transcribed sequences +47 to +70. After primer extension³⁶, products were separated on a 8% denaturing polyacrylamide gel followed by autoradiography for 48 h with a screen; solid arrow, LTL1 extension product; open arrow, RSVCAT extension product.

domains before ligand binding, either directly or by contacting proteins that interact with the intact receptor; it is intriguing that the polypeptide segment between the nonfunctional derivatives (1–615) and constitutive derivatives (1–605 and 1–595) is similar (~50%) to a corresponding segment of the human oestrogen receptor^{28,29}.

In general, little is known about how bound ligands modulate the activities of transcriptional regulatory proteins, but substantial information has been obtained from three-dimensional structure and genetic analyses of two proteins from *Escherichia coli*, the cyclic AMP receptor^{30,31} and the tryptophan repressor³².

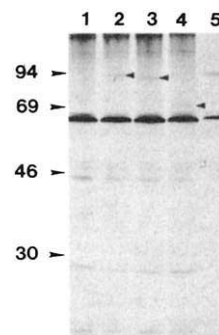


Fig. 4 Glucocorticoid receptor protein expression in transiently transfected cells. COS-7 cells (10^6) were transfected (see Fig. 3 legend) with control plasmid pSV7d (lane 1), or with plasmids encoding receptor derivatives 1–795 (lane 2), 1–768 (lane 3), or 1–556 (lane 4); 40 h after transfection, the cultures were labelled for 16 h with a mixture of 35 S-methionine and 35 S-cysteine (1 mCi). Lane 5, similarly labelled proteins from G10 cells, an HTC cell derivative containing stably transfected and expressed full-length (1–795) receptor¹². Cell extracts were subjected to immunoprecipitation with receptor-specific monoclonal antibodies¹⁷; precipitates were fractionated by SDS-gel electrophoresis, followed by autoradiography. Positions of receptor species are indicated by small arrowheads; migration of size markers is shown at the left.

Interestingly, in both cases, the results are consistent with the induction model (Fig. 2*a*), rather than the derepression mechanism proposed to mediate transformation of the glucocorticoid receptor. For both the *E. coli* proteins, ligand binding appears to induce alterations in the structural relationships of particular α -helical domains, leading ultimately to the formation of 'helix-turn-helix' motifs³³ capable of specific DNA recognition. The glucocorticoid receptor DNA-binding domain (Fig. 1; S.R. and K.R.Y., in preparation) lacks a discernable helix-turn-helix motif; instead, the receptor may contain finger-like protein loops constrained by tightly coordinated zinc ions²², as proposed³⁴ for transcription factor TFIIIA. Thus, the induction mechanism may be particularly suitable for transmission of structural changes through interhelical interactions that eventually produce functional motifs that themselves depend upon specific interhelical orientations. In contrast, it may be more difficult to transmit structural alterations into other types of protein domains, especially those conformationally constrained by bound cofactors; consequently, regulation might be more readily imposed simply by masking the otherwise functional structures.

The deletion mutants described here have delineated a region of the glucocorticoid receptor that will be interesting for detailed analysis. With additional genetic, molecular and structural studies, it should be possible to obtain a clear view of the mechanism by which small physiological regulators such as steroid hormones modulate specific genes transcription by facilitating changes in the structure of their receptors.

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Activation of transcription by two factors that bind promoter and enhancer sequences of the human metallothionein gene and SV40

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Genetic analysis of eukaryotic transcriptional promoters has revealed that protein-coding genes often contain a complex array of *cis*-control elements consisting of upstream activator sequences and enhancer elements¹⁻⁵. The metallothionein genes provide a useful example for dissecting the action of multiple interspersed control elements that govern both basal level and regulated expression in animal cells⁶⁻⁸. The human metallothionein (*hMTII_A*) promoter has been analysed in detail and found to contain no less than five distinct control elements in the 5' flanking regions of the gene that mediate specificity and regulation of transcription^{9,10} (Fig. 1). These different control elements can be functionally subdivided into two categories: basal and induced elements. There are several distinct basal recognition sequences, which include a TATA-box, a GC-box, and at least two basal level enhancer (BLE) sequences, that function like classical enhancer

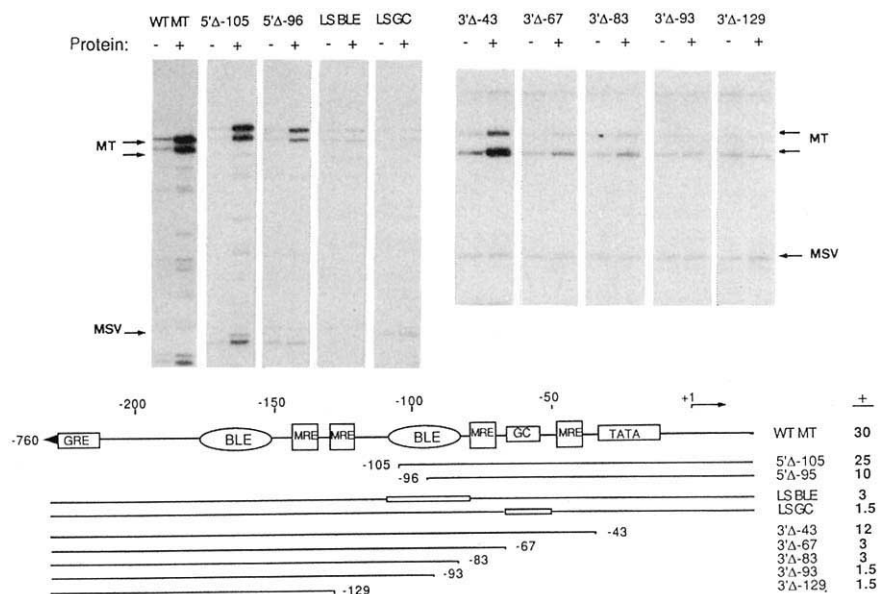
elements¹⁰⁻¹². The *hMTII_A* gene also responds to induction by heavy metals and by steroid hormones through the action of metal regulatory elements (MRE) and glucocorticoid responsive elements (GRE)⁹. Here we report the identification of two cellular DNA-binding proteins that interact selectively with sequences governing the basal level expression of *hMTII_A*. One of these factors is a novel activator protein (API) that interacts with sequences in the BLE of *hMTII_A* and also binds to a site within the 72-base pair (bp) repeats of the simian virus 40 (SV40) enhancer region. The second protein has been purified to homogeneity and shown to be transcription factor Sp1 which recognizes and binds to a single GC-box element within the *hMTII_A* promoter.

A powerful approach for studying the relationship between *cis*-control elements in the *hMTII_A* promoter and potential cellular transcription factors is to complement *in vivo* promoter analysis with *in vitro* transcription reactions^{13,14}. We have therefore reconstituted transcription of *hMTII_A* by using partially purified endogenous RNA polymerase II, general initiation factors and a fraction designated S-300, which contains auxiliary proteins required to confer promoter selectivity. A collection of wild-type and mutant templates containing various portions of the *hMTII_A* promoter fused to the structural coding regions of a heterologous gene, the *TK* gene of herpes simplex virus (HSV-*TK*), was used to direct RNA synthesis *in vitro*^{9,10}. The level and accuracy of RNA synthesized *in vitro* was determined by primer extension and the efficiency of transcription from each *hMTII_A* template was measured relative to transcription from a truncated murine sarcoma virus (MSV) promoter as a control. The MSV template contains a TATA box but lacks upstream regulatory elements of the large terminal repeat (LTR) and thus does not respond to promoter-specific factors.

A template (wild-type) that contains 5' flanking sequences extending 760 bp upstream of the initiation site (nucleotide position +1) directs transcription at a low but detectable level in the absence of the S-300 fraction (Fig. 1). However, in the presence of S-300, transcription is stimulated 5- to 10-fold. As expected, transcription of the control MSV template is not enhanced by the addition of the S-300 fraction. A second template containing sequences to nucleotide -105 also exhibits low activity in the absence of the S-300 fraction and a 10-fold enhancement of transcription in the presence of this fraction. In contrast, deletion of nine additional nucleotides renders the 5' deletion mutant Δ -96 significantly less responsive to factor-dependent activation, although stimulation is not entirely abolished (Fig. 1). Analysis of 3' deletion mutants indicates that sequences downstream of -43 are not important for directing transcription *in vitro* when the TATA-box element and initiation sites derived from the heterologous *TK* gene are present. However, removal of 3' sequences to -67 or -83 significantly reduces the S-300 fraction-dependent activation of transcription from the *hMTII_A* promoter. Further 3' deletions of sequences to -93 and -129 essentially abolish any S-300 factor-dependent transcription.

To determine the nature and number of distinct promoter elements in the -105 to -43 region, we have used two linker-scanning mutations that specifically introduce clustered base substitutions in either the 5' or 3' portion of the upstream control region mapped by deletion analysis. A linker-scanning mutation introduced near the 5' border (LS-BLE) interrupts by clustered base substitutions sequences in one of the basal level enhancer (BLE) elements identified *in vivo* by transient transfection assays¹¹. The linker-scanning mutation introduced near the 3' border (LS-GC) interrupts a GC-box motif that is a perfect match for sequences recognized by the previously identified cellular transcription factor, Sp1 (refs 4, 13-16). Transcription of LS-BLE is at normal background levels in the absence of the S-300 factors but, unlike in the wild-type or Δ 5'-105, the presence of added S-300 components only activates transcription to a small degree, about twofold. Similarly, LS-GC, in which transcription shows little if any response to the S-300 fraction,

Fig. 1 Analysis of RNA synthesized from wild-type and mutant *hMTII_A/TK* promoter fusions in an *in vitro* reconstituted transcription reaction. Above, labelled primer extension products obtained in the presence (+) or absence (–) of promoter-specific transcription factors, using a promoter fusion (WTMT) or mutant derivatives of it. Arrows, expected sizes of accurately initiated transcripts of the *TK* gene (MT) controlled by the promoter constructs and a gene not controlled by it for comparison (MSV). Below, schematic diagram of the promoter region of various constructs; horizontal lines show sequences present in each. BLE, basal level enhancer; GC, GC-box element centred at –63. Clustered base substitutions within LS-BLE and LS-GC are indicated by thin rectangles. Values in the right-hand column are the relative efficiency of *in vitro* transcription directed by various wild-type and mutant templates in the presence of the S-300 protein fraction.



Methods. Wild-type template was derived by fusing a fragment of *hMTII_A* (–770 to +70) to the HSV-*TK* structural coding sequences at position (+50) as described elsewhere (pHSI/TK, ref. 10). Constructs 5'Δ-105 and –96 were generated from pHSI/TK by *Bal31* deletion. The 3'Δ templates were constructed by fusing 3' deletions of *hMTII_A* to the *Bam*HI site of TKΔ5'–46 (ref. 28). LS-BLE was generated by replacing the chloramphenicol acetyl transferase (*cat*) structural sequences from pX-LS110/80 (ref. 10) with the *Bgl*III–*Eco*RI fragment derived from pTK^{28,29}. LS-GC was constructed as described previously (LS ES, ref. 10). The structure of each hybrid template was confirmed by direct DNA sequence analysis. The *in vitro* reconstitution reaction contains supercoiled template (100 ng), and internal control plasmid 5'Δ-32 MSV (25 ng), incubated with partially purified RNA polymerase II and Sp2¹³ in the presence (+) or absence (–) of a Sephacryl S-300 gel filtration fraction enriched for promoter-specific transcription factors¹⁶. All transcription reactions were incubated for 30 min at 30 °C. Preparation of RNA and the method of primer extension were as described¹³. Labelled primer extension products were analysed by electrophoresis on a 8% polyacrylamide sequencing gel with DNA sequence markers. Autoradiographic exposures were obtained after 12 h at –70 °C with intensifying screen. The primer extension product for the wild-type transcript is 3–5 nucleotides shorter than all the other transcripts derived from *hMTII_A* because the position of the promoter fusion to the *TK* body is closer to the *TK* gene than in the other cases.

behaves much like a 3' deletion lacking the GC-box sequences (Δ3'–93). The bimodal response of the promoter suggests that there are at least two elements between –105 and –43, both of which contribute to the S-300 factor-dependent activation of *hMTII_A* transcription *in vitro*. Note that mutations in the BLE element, known to function as an enhancer *in vivo*, have a very significant influence on *in vitro* transcription reactions and that BLE-dependent activation requires factors present in the S-300 fraction. We conclude that the multiple *cis*-control elements identified through *in vivo* mapping studies are also functional *in vitro*, allowing us to test directly for cellular proteins in the S-300 fraction that recognize and bind to specific *cis*-control elements in the *hMTII_A* promoter.

The ability of DNA-binding proteins in transcriptionally active S-300 fractions to interact with *hMTII_A* promoter sequences was determined by DNase I footprint analysis. Double-stranded DNA probes containing the upstream region of the *hMTII_A* promoter were end-labelled, incubated with various amounts of the S-300 fraction in the presence of nonspecific competitor DNAs, subjected to partial DNase I digestion, and separated alongside size markers on a denaturing polyacrylamide gel. The DNase I protection pattern obtained with wild-type *hMTII_A* promoter probes is shown in Fig. 2a. Three regions of the promoter were protected from DNase I digestion as the concentration of S-300 proteins was increased. At low concentrations, complete protection is seen in the region (footprint I) which coincides with the GC-box element of the promoter. Partial protection of regions II and III occurs at low protein concentrations and complete protection only at higher levels of protein. Direct comparison of binding regions II and III to DNA sequence markers (data not shown) indicates that these protected areas overlap with the proximal and distal BLE elements upstream of the *hMTII_A* promoter^{10,11}.

Sp1 purified to homogeneity has been used to determine whether the GC-box element found in *hMTII_A* is indeed recognized by Sp1. Purified Sp1 protein isolated by sequence-specific DNA affinity chromatography^{15,16} was used in a footprint reaction, and the pattern of protected sites was compared directly with protection conferred by partially purified S-300 proteins (Fig. 2b). In the presence of pure Sp1, only the GC-box region was protected from DNase I digestion. These findings demonstrate that Sp1 binds the GC-box of the *hMTII_A* promoter, and suggest that a second distinct binding activity is responsible for recognizing and interacting with sequences within the BLE. We have designated this cellular factor, which binds to the promoter proximal BLE, Activator Protein-1 (AP-1).

DNA-binding proteins that recognize upstream control elements of the *hMTII_A* promoter are likely to be factors that activate transcription *in vitro* and *in vivo*. To investigate the correlation between specific DNA-binding activities and transcriptional activation, we used linker-scanning mutants in the BLE and GC elements. We have carried out DNA binding reactions (Fig. 2c) with the same linker-scanning mutants which previously were shown to hamper transcription severely (see Fig. 1). DNA probes derived from LS-BLE, and LS-GC were 5' end-labelled at the *Nco* I (+71) and *Xpa* I (+73) sites, respectively, and the S-300 fraction, containing both Sp1 and AP-1, was used in DNase I footprint reactions. The pattern of protected regions in LS mutant DNAs was then compared directly to footprints obtained with wild-type template. The clustered base substitutions in the BLE region alter and diminish binding and protection of this region by AP-1, but have no effect on Sp1 interactions at the GC-box. In contrast, LS-GC is unable to bind Sp1 whereas interactions of AP-1 with the BLE region occur unimpaired. These data are consistent with specific protein-DNA interactions at the BLE and GC elements being func-

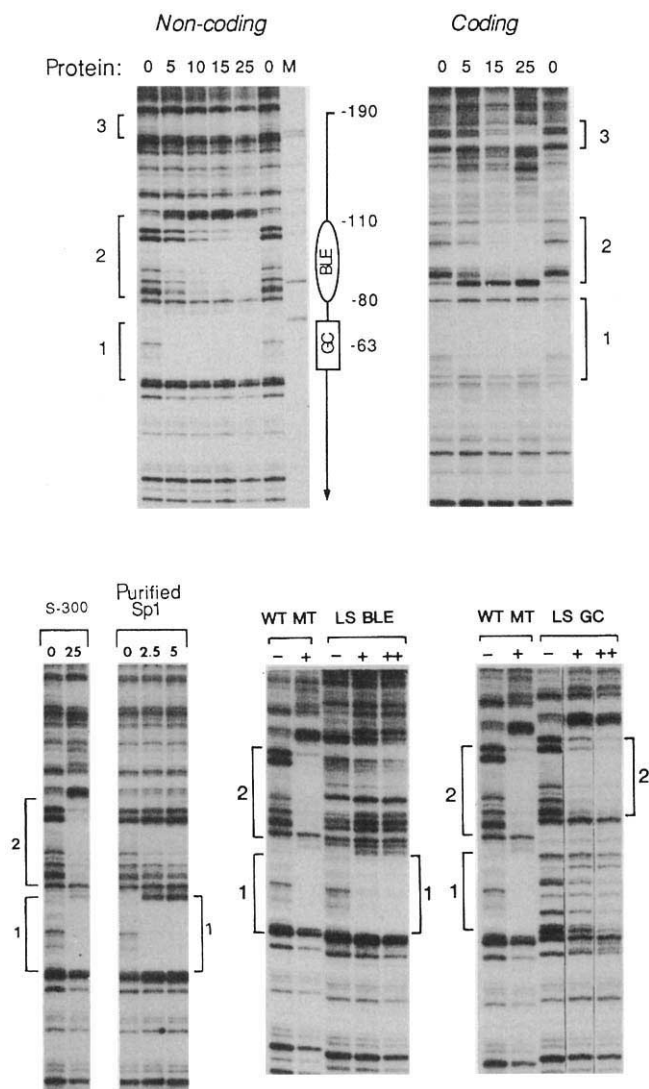


Fig. 2 Analysis of DNA-binding protein interactions with *hMTIIA*. **a**, DNase I footprints of wild-type *hMTIIA* DNA. Non-coding strand probe (840 bp), pHSI (ref. 10) was 5' end-labelled at the *Bam*HI site (+73 relative to the start site of transcription) using [γ - 32 P]ATP and polynucleotide kinase. Coding-strand probe was prepared by labelling the *Bam*HI cleavage site in pHSI with [α - 32 P]NTPs and avian myeloblastosis virus (AMV) reverse transcriptase. Numbers above each lane, amount (μ l) of S-300 protein used in the footprint reaction; M, DNA size markers. DNase I footprint reactions were as described³⁰. The three binding sites upstream of the *hMTIIA* regulatory region that are protected from digestion by DNase I are indicated by brackets 1, 2 and 3. Relative positions of protected regions and specific *cis*-regulatory control elements (BLE and the GC-box) previously mapped *in vivo* are also shown. **b**, Binding of purified Sp1 to the *hMTIIA* promoter. Transcription factor Sp1 was purified to homogeneity from HeLa cells as described^{15,16}. Left-hand lanes, the pattern of DNase I cleavage in the noncoding-strand probe of *hMTIIA* in the presence S-300 protein (0–25 μ l). The remaining lanes depict the pattern of protection in the absence (0) or presence (2.5, 5) of purified Sp1. The brackets on the left mark protected regions (1, GC; 2, BLE). **c**, Interaction of AP-1 and Sp1 with linker-scanning mutants of *hMTIIA*. DNase I footprint protection of LS-BLE and LS-GC were carried out with S-300 protein fractions and 5' end-labelled probe DNA derived from *Bam*HI cleavage of LS-BLE and GC template DNAs. Left panel, the pattern of protection observed with either wild-type template (left two lanes) or LS-BLE template (right three lanes) in the absence (–) or presence (+, ++) of S-300 protein. Right panel, DNase I footprint protection observed with wild-type template (left two lanes) or LS-GC template (right three lanes) in the absence (–) or presence (+, ++) of S-300 protein. Brackets, boundaries of the protected region (1, GC; 2, BLE).

tionally important for promoter activation, because the behaviour of these mutants in DNA-binding assays correlates with the results obtained for transcription. In addition, these findings demonstrate that binding of Sp1 to the GC element and the binding of AP-1 to the BLE element can occur independently of each other, albeit at closely adjacent sites.

Alternatively, involvement of a DNA-binding protein in transcription can be examined using *in vitro* reconstituted reactions to measure stimulation of RNA synthesis by purified proteins. Sp1 purified to homogeneity could be tested directly for transcriptional activation of *hMTIIA* *in vitro*. In the presence of partially purified RNA polymerase II and general initiation factors (Fig. 1 legend), transcription of both the wild-type and mutant templates (Δ 3'-67 and LS-GC) gives rise to a low basal level of transcription (Fig. 3). On addition of purified Sp1, there is a 5- to 6-fold enhancement of transcription directed by the wild-type template. By contrast, templates derived from Δ 3'-67 and LS-GC, which lack a functional GC-box, fail to respond to added Sp1. These reconstitution reactions and the more extensive analysis of mutant templates using crude extracts (Fig. 1) firmly establish that transcription factor Sp1 activates RNA synthesis of the *hMTIIA* gene *in vitro*.

Identification of AP-1 as a cellular DNA-binding protein that recognizes specific sequences in the BLE of *hMTIIA* prompted us to test for other transcriptional activator elements that interact with the same factor. In particular, AP-1 might recognize *cis*-

control sequences that have been associated with other enhancer elements *in vivo*^{17–20}. Inspection of the footprint-protected region revealed a stretch of DNA containing the sequence TGANTCA that is also present in the 72-bp enhancer elements (at nucleotide 115 and 190) of the SV40 genome. We therefore tested binding of AP-1 present in the S-300 fractions to sequences in the SV40 72-bp elements. Footprint protection of SV40 DNA probes containing two copies of the 72-bp enhancer element reveals a complex pattern of binding sites that include the GC-boxes of the 21-bp repeats, many protected regions in the 72-bp elements, and at least one binding site just outside the 5' distal 72-bp repeat. Putative AP-1 recognition sequences within SV40 DNA are adjacent to Sp1 binding site VI (nucleotide 115) and at the equivalent 3' proximal position (nucleotide 190) of the distal 72-bp repeat (Fig. 4a). Because the S-300 fraction contains several sequence-specific DNA binding activities and the 72-bp elements consist of several distinct recognition sites^{18–20}, it is not possible to determine if the same factor, AP-1, binds to both the SV40 72-bp element and the *hMTIIA* BLE region by direct footprint analysis. Using footprint competition assays, we have demonstrated that at least one common binding factor other than Sp1 recognizes sequences in both SV40 and *hMTIIA*.

DNase I footprint reactions using end-labelled *hMTIIA* probe DNA were assayed in the presence of various amounts of a restriction fragment containing only the 72-bp repeats of SV40.

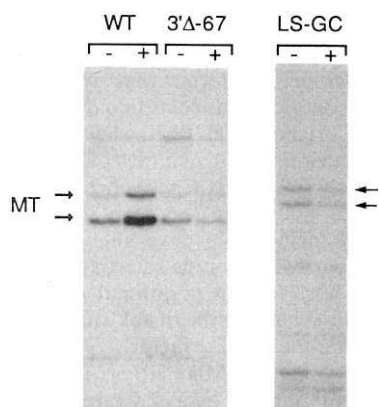


Fig. 3 Activation of *hMTII_A* transcription by Sp1. The *in vitro* reconstituted transcription reaction contains supercoiled DNA template (100 ng) incubated with partially purified HeLa RNA polymerase II and initiation factor Sp2 (ref. 13) in the presence (+) or absence (-) of purified Sp1^{15,16}. The wild-type and mutant templates ($\Delta 3'-67$, LS-GC) were assayed as in Fig. 1. Arrows labelled MT indicate the primer extension products expected for accurate initiation of RNA transcription on the *hMTII_A* templates.

If the AP-1 factor present in the S-300 interacts with and binds to both the BLE and regions within the 72-bp repeats of SV40, then the characteristic pattern of protected sequences in the BLE should be competitively inhibited. The experiments of Fig. 4b demonstrate that a molar excess of the SV40 72-bp repeats effectively competes for binding of a factor to the BLE element, but does not interfere with the Sp1 binding to the GC-box of *hMTII_A*. As expected, in the presence of competitor DNA containing only the 21-bp elements of SV40, which contain six copies of the GC-box element, binding of Sp1 to *hMTII_A* is significantly inhibited. We have also found that a synthetic oligonucleotide derived from the *hMTII_A* proximal BLE sequences containing the AP-1 recognition site specifically competes with the AP-1 binding site in the 72 bp region of SV40 but not with the other binding activities observed in the S-300 fraction (M. Imagawa and M.K., unpublished data). These *in vitro* results confirm and extend previous *in vivo* template competition studies that suggested *hMTII_A* and SV40 share some common recognition elements²¹.

Thus, HeLa cells contain at least two distinct transcription factors, Sp1 and AP-1, that recognize and bind selectively to sequences in both the SV40 and *hMTII_A* promoters. Of these two cellular transcription factors, the newly identified activator protein AP-1 is of particular interest because it binds to sequences in both the metallothionein II_A gene (the BLE) and the SV40 genome (72-bp repeats) that have been associated with enhancer function^{17-20,21}, and can stimulate *hMTII_A* transcription *in vitro*. We have also demonstrated the involvement of transcription factor Sp1 in *hMTII_A* RNA synthesis²²⁻²⁵. Although, the *hMTII_A* promoter (unlike the tandemly-arrayed Sp1 binding sites in SV40) contains only a single high-affinity binding site, mutations restricted to the GC-box of *hMTII_A* can still cause severe impairment of promoter function. Perhaps the dominant influence of Sp1 in the *hMTII_A* promoter is due in part to the location of the strong consensus GC-box close to the TATA box region, flanked by sequences that have been shown genetically to influence metal regulation and enhancer function. The mechanism by which Sp1 potentiates transcription in either SV40 or *hMTII_A* is not yet understood. One possibility is that the Sp1 protein^{15,16} (relative molecular mass 105,000) not only binds its DNA recognition sequences^{24,25}, but also interacts productively with other components of the transcription apparatus through direct protein-protein contact. The failure of AP-1 to stimulate transcription of the mutant LS-GC, which prevents binding of Sp1 but not AP-1, suggests that an interac-

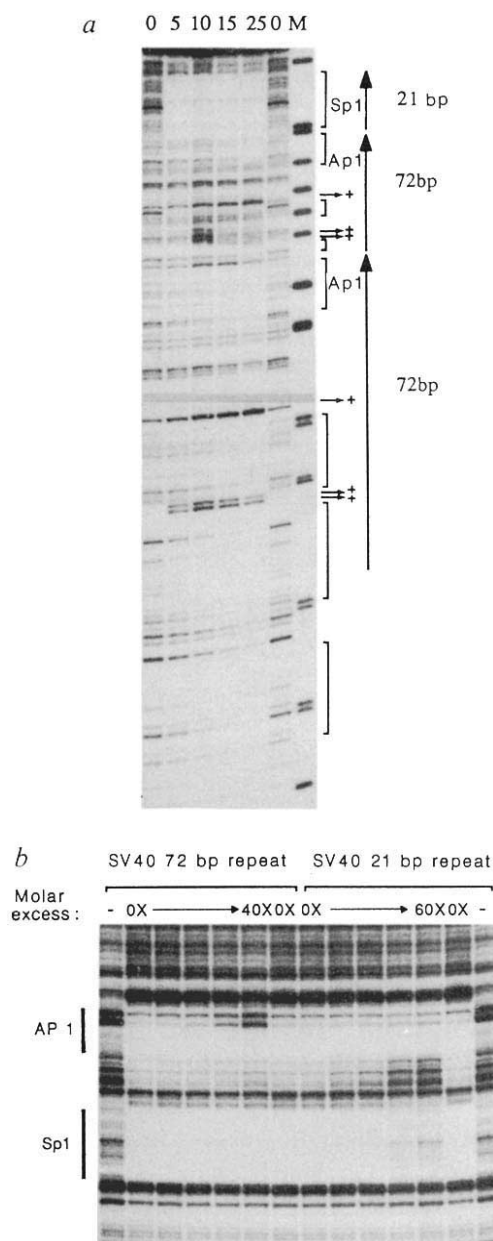


Fig. 4 Interaction of AP-1 with sequences in the 72-bp repeats of the SV40 enhancer element. *a*, DNase I footprint protection of the SV40 control region. Different amounts (0, 5, 10, 15, 20 μ l) of the S-300 fraction enriched for Sp1 and AP-1 factors were used to protect sequences in the 21-bp GC-box elements and the 72-bp repeats of SV40. DNA footprint probes end-labelled at the *Hpa*II site were prepared as described³⁰. Brackets, boundaries of regions protected from DNase I digestion by various DNA-binding proteins present in the S-300 fraction, including Sp1 and AP-1. Putative AP-1 sites have been tentatively identified on the basis of sequence homology to the proximal BLE of *hMTII_A*. Horizontal arrows marked +, locations where enhanced cleavages by DNase I was observed in the presence of S-300 fraction; vertical arrows, the 21-bp and 72-bp repeat elements of the SV40 control region; M, size markers. *b*, Binding of Sp1 and AP-1 to *hMTII_A* in the presence of competitor DNA fragments derived from the SV40 21-bp or 72-bp repeats. DNase I footprint reactions were carried out with fragments of *hMTII_A* DNA, 5' end-labelled at the *Bam*HI site of pHSI as in Fig. 3. Protection from DNase I digestion was determined in the presence of S-300 protein (25 μ l) and varying amounts (0 to 40-fold molar excess) of competitor DNA derived from either a fragment of SV40 containing only the 72-bp repeat sequences, or a fragment containing only the 21-bp repeat sequences. The left and right lanes show the pattern of DNase I cleavages in the absence of any added S-300 protein.

tion of AP-1 with Sp1 may be required to transmit its effect over a distance. Our studies provide biochemical evidence that two *trans*-activator proteins, one which interacts with enhancer elements and another that binds to proximal promoter sequences, probably function coordinately to direct transcription. One consequence of such a mechanism is that a given activator element may be used by a variety of different genes in conjunction with other elements to confer transcriptional specificity^{4,5,26,27} and participate in a complex regulatory network. Understanding the interrelationships of different activator proteins that bind to DNA and their mechanisms for potentiating initiation of transcription requires the biochemical purification and characterization of individual components of the transcriptional machinery. This task has recently been made less difficult by the development of efficient purification procedures^{15,16}, and it should now be feasible to purify transcription factors such as AP-1 to homogeneity and characterize their biochemical properties.

It is evident from genetic analysis that, in addition to the GC-box and proximal BLE, the *hMTII_A* promoter contains other

cis-regulatory elements that play an important role in transcriptional specificity (Fig. 1). So far, no sequence-specific DNA-binding protein has been detected that interacts with MREs, but *hMTII_A* also contains a glucocorticoid responsive element (GRE), upstream of the distal BLE, at which glucocorticoid receptor binds⁹. Previous *in vivo* studies of *hMTII_A* have identified two apparently related BLE sequences that act in concert with the MREs to potentiate the high levels of expression induced by heavy metals. We have shown that the cellular protein AP-1 recognizes and binds selectively to the promoter-proximal BLE of *hMTII_A* but additional experiments will be required to determine whether the distal BLE also interacts with AP-1 or with a different cellular factor.

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Vanadium K-edge X-ray absorption spectrum of the VFe protein of the vanadium nitrogenase of *Azotobacter chroococcum*

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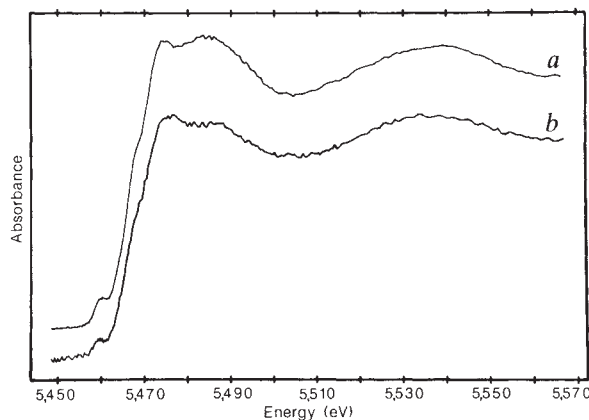


Fig. 1 Vanadium K-edge and XANES of (a) $[\text{Me}_4\text{N}]\text{-}[\text{VFe}_3\text{S}_4\text{Cl}_3(\text{DMF})_3]$ and (b) Ac1^* .

The free-living, nitrogen-fixing bacterium *Azotobacter chroococcum* has recently been shown to contain two nitrogenase systems¹. One of these is the well-characterized molybdenum-containing enzyme^{2,3} and the other, which is encoded by different genes, contains vanadium. Both enzyme systems consist of two proteins: an iron protein and a molybdenum- or vanadium-containing protein which also contains iron. The vanadium-containing nitrogenase protein, Ac1^* , has a relative molecular mass $\approx 210,000$ and contains 2 vanadium atoms, 23 iron atoms, and 20 acid-labile sulphide ions per mole⁴. X-ray absorption spectroscopy has been extensively used to probe the environment of molybdenum in the MoFe protein⁵. The molybdenum is closely associated with iron and sulphur in an iron-molybdenum cofactor, FeMoco, which is probably the N_2 -binding site of the enzyme⁶. We report here vanadium X-ray absorption data of the VFe protein, the first such report for any vanadium-containing protein, and compare them with those from a recently synthesized VFeS cluster compound⁷. The results suggest

that the vanadium in this protein is present in a vanadium-containing cofactor analogous to FeMoco. We conclude that substitution of vanadium for molybdenum probably accounts for the different substrate specificities of the two nitrogenases.

Ac1^* was isolated from a strain of *A. chroococcum*, MCD1155, in which the genes (*nifHDK*) encoding the molybdenum nitrogenase polypeptides had been deleted, and was purified as described¹. The specific activity was 1,536 nmol H_2 evolved per min per mg protein. The protein, in 50 mM HEPES, pH 7.4, containing 100 mM NaCl and 2 mM $\text{Na}_2\text{S}_2\text{O}_4$, was concentrated in a Minicon concentrator (Amicon) to 255 mg ml^{-1} with a final vanadium concentration of ~ 2 mM. The cluster compound $[\text{Me}_4\text{N}][\text{VFe}_3\text{S}_4\text{Cl}_3(\text{DMF})_3]$, (DMF = *N,N*-dimethylformamide), which contains a VFe_3S_4 cubane-like cluster and invol-

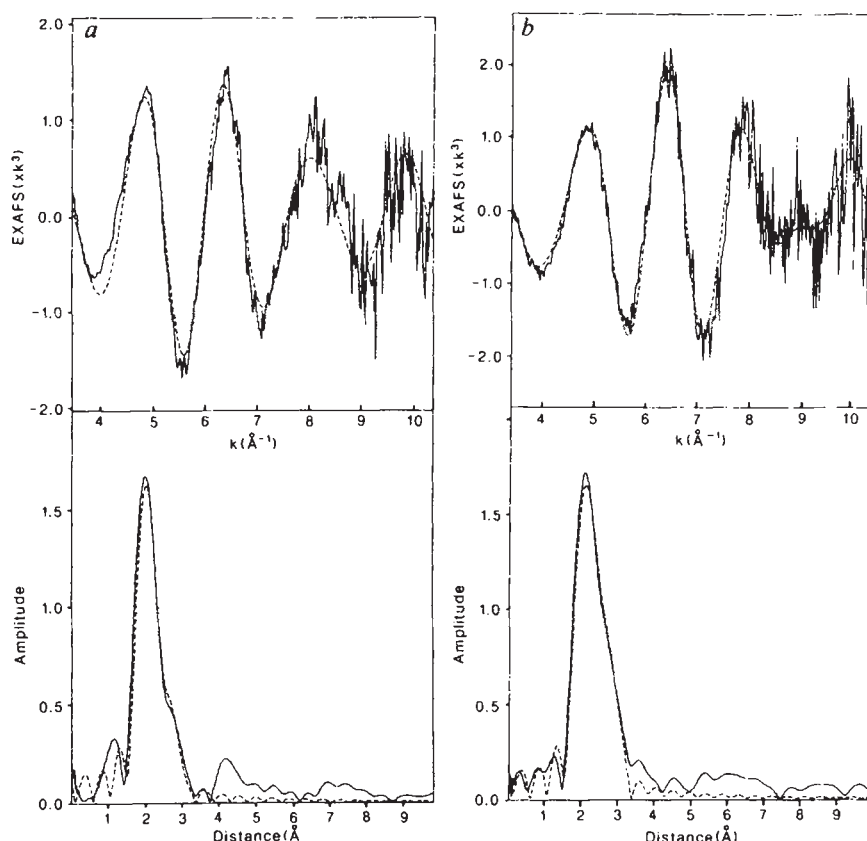


Fig. 2 EXAFS (χk^3) data (top traces) with (lower traces) Fourier transform for (a) $[\text{Me}_4\text{N}][\text{VFe}_3\text{S}_4\text{Cl}_3(\text{DMF})_3]$ and (b) AcI^* . Solid line, experimental data; broken line, simulation using the data of (a) Table 1 or (b) Table 2.

ves the additional coordination of vanadium by three DMF molecules external to the cluster, was prepared as reported⁷ and studied as a DMF solution with vanadium concentration ~ 10 mM. Both samples were stored in liquid nitrogen after anaerobic loading into cells.

X-ray absorption spectra were recorded in fluorescence mode on EXAFS station 8.1 at the Daresbury Synchrotron Radiation Source operating at 2 GeV and an average current of 150 mA, a windowless ionization chamber being used to record the incident intensity and a single NaI scintillation detector with a titanium foil the fluorescent intensity. The use of a double crystal Si(III) monochromator minimized harmonic contamination⁸ but crystal glitches limited the accessible data range to approximately 400 eV beyond the edge; 15 scans were recorded and averaged for both the protein and the cluster. During data collection a liquid nitrogen cryostat was used to maintain a sample temperature of ~ 80 K. Data analysis employed the single-scattering spherical-wave method for EXAFS calculation and phaseshifts derived from *ab initio* calculations as described elsewhere⁹⁻¹¹.

Figure 1 shows the vanadium K-absorption edge and XANES (X-ray absorption near edge structure) of both the cluster and AcI^* . The pre-edge feature ($5,460 \pm 1$ eV) and the edge inflection ($5,467 \pm 1$ eV) are almost identical in position and intensity in both systems, suggesting the same oxidation state and electronic configuration for the vanadium in each case. The position of the pre-edge features, some 3 eV higher in energy than for vanadium metal, indicates an oxidation state of between V(II) and V(IV)¹², consistent with the view expressed by Kovacs and Holm in respect of the VFeS cluster⁷. Comparison of these edge profiles with corresponding data for a range of well-defined vanadium compounds¹² implies octahedral coordination of the vanadium, as established by X-ray crystallography⁷ for $[\text{VFe}_3\text{S}_4\text{Cl}_3(\text{DMF})_3]^-$. Thus, the pre-edge feature is assigned to a 1s to 3d transition that is quadrupole allowed but dipole forbidden in octahedral symmetry and is therefore weak.

In contrast, the XANES regions (≤ 50 eV from the edge, Fig. 1) of the cluster and the protein are not identical. As this spectral region is sensitive to multiple scattering contributions it can provide geometric information, for example by qualitative comparison with other vanadium systems. Thus, the differences in the XANES are presumed to reflect differences in the detailed geometry about vanadium in these two systems.

The vanadium K-edge EXAFS data for $[\text{Me}_4\text{N}][\text{VFe}_3\text{S}_4\text{Cl}_3(\text{DMF})_3]$ are shown in Fig. 2a, together with a theoretical simulation using the parameters given in Table 1, where

Table 1 Comparison of EXAFS parameters and X-ray crystallographically determined (XRD) distances for $[\text{Me}_4\text{N}][\text{VFe}_3\text{S}_4\text{Cl}_3(\text{DMF})_3]$

Atom	N	EXAFS parameters		XRD
		R (Å)	$2\sigma^2$ (Å ²)*	R (Å)
O	3.0	2.12	0.001	2.112–2.145
S	3.0	2.35	0.029	2.331–2.340
Fe	3.0	2.73	0.031	2.771–2.781

$E_0 = 17.20$ eV; energy range, 26–393 eV. Errors in bond distances are considered to be ± 0.03 Å. XRD, data taken from ref. 7.

* Debye-Waller factor.

the corresponding crystallographically determined distances are also presented⁷. The V–O and V–S distances determined by EXAFS are in good agreement with the crystallographic data (within the estimated error in the EXAFS determined distances of ± 0.03 Å). The V–Fe distance obtained by EXAFS (2.73 Å) is approximately 0.05 Å short, presumably because of some slight inadequacies in the calculated phaseshifts.

The extraction of accurate coordination numbers from the EXAFS of $[\text{Me}_4\text{N}][\text{VFe}_3\text{S}_4\text{Cl}_3(\text{DMF})_3]$ proved difficult because, at the particular distances involved, the backscattering contributions from the oxygen and sulphur shells are in phase over almost all the available data range. This results in high correla-

tions between the coordination numbers and Debye-Waller factors of these two shells, so these parameters are not independent and any change in one will significantly affect the other values on refinement. In this case (where the coordination numbers are known) least squares refinement of the Debye-Waller factors (see Table 1) resulted in a dominant contribution from V-O backscattering. A series of simulations were produced where the total number of oxygen plus sulphur ligands was held at 6 and the O:S ratio varied (only integer values were considered for these coordination numbers and 3 iron neighbours were used throughout); for each ratio, the vanadium-neighbour distances and their associated Debye-Waller factors were refined. This procedure showed that the data could be fitted quite well with 6 oxygen and 3 iron atoms at distances within the limits set above, but the quality of the fit was improved slightly yet significantly by the inclusion of backscattering from sulphur atoms. Consistent with the crystallographic results, the best interpretation of the experimental data (Fig. 2a) required 3 oxygen plus 3 sulphur atoms.

The same approach was adopted in the analysis of the EXAFS data for Ac1* which are shown in Fig. 2b, together with the Fourier transform of the data and the best theoretical fit using the parameters given in Table 2. The EXAFS of Ac1* (Fig. 2b)

Table 2 Parameters used to simulate the EXAFS of Ac1*

Atom	N	R (Å)	2 σ^2 (Å ²)†
O‡	4.0	2.14	0.001
S	2.0	2.32	0.030
Fe	3.0	2.69§	0.014

$E_0 = 17.20$ eV; energy range, 25–398 eV. Error in coordination number considered to be ± 1 ; error in bond distance considered to be ± 0.03 Å.

† Debye-Waller factor.

‡ O, N or C are all possible first shell ligands.

§ Value before correction by addition of 0.05 Å (see text).

resembles that of the VFeS cluster compound (Fig. 2a) and this similarity implies a similar environment for the vanadium. Furthermore, the EXAFS data for Ac1* can be simulated successfully with backscattering contributions from oxygen, sulphur, and iron, the vanadium-neighbour distances being determined as 2.14, 2.32, and 2.69 Å, respectively (all ± 0.03 Å). The V-Fe distance is considered to be ~ 0.05 Å shorter than the true value, given the results for the VFeS cluster compound. As in the interpretation of the EXAFS of this compound, the inclusion of backscattering from oxygen and iron atoms is vital to the quality of the fit and the addition of sulphur atoms significantly improves it. For a total of 6 (oxygen plus sulphur) ligands, consistent with the octahedral coordination deduced from the edge profiles, the best fit was obtained with 4 ± 1 oxygen and 2 ± 1 sulphur atoms. Refinement of the number of iron atom neighbours gave values of between 2.5 and 3.0, for a total of 6 inner shell ligands; these values imply that 3 rather than 2 sulphur atoms are bound to the vanadium.

The major differences in the vanadium K-edge EXAFS of the cluster compound and Ac1* can be accounted for by a reduced Debye-Waller factor and a slightly shorter V-Fe distance in Ac1* (Tables 1 and 2); the former difference suggests a more ordered structure around the vanadium in the protein than in the VFeS cluster compound. The apparent change in the V-Fe interaction (and the possible difference in inner-shell coordination numbers) may account for the differences observed in the XANES region for the VFeS cluster and Ac1* (Fig. 1).

The similarity between the environment of vanadium in Ac1* and of molybdenum in FeMoco⁵ is interesting, suggesting the presence of a vanadium-containing cofactor in Ac1*. By analogy this would include, or form part of, the enzyme's active site. Substitution of vanadium for molybdenum would account for the different substrate specificities of the two nitrogenases¹.

Refinement of the analysis presented above should be possible by extending the range and quality of the data for Ac1* and by studying further model compounds.

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Errata

A protein on *Plasmodium falciparum*-infected erythrocytes functions as a transferrin receptor

Mario H. Rodriguez & Michele Jungery

Nature **324**, 388–391 (1986).

IN this letter Figs 1 and 2 were transposed. The legends are correct as printed.

The essential light chains constitute part of the active site of smooth muscle myosin

Yoh Okamoto, Takamitsu Sekine, Jean Grammer

& Ralph G. Yount

Nature **324**, 78–80 (1986).

FIGURES 1 and 2 in this letter were transposed. The legends are correct as printed.

Corrigendum

Searching potential energy surfaces by simulated annealing

L. T. Wille

Nature **324**, 46–48 (1986).

SINCE publication of this letter a sixteen-particle structure has been discovered which is nearly degenerate with that given in Table 1, but has a marginally lower energy ($E = 92.912$). This structure was obtained using exactly the same algorithm and parameters as described in the paper. Due to statistical fluctuations, the five simulations originally reported ended up in the 4^4 configuration ($E = 92.920$), whereas four out of five new runs converged to the new 13^5 structure. It consists of four charges, occupying the vertices of a tetrahedron, and four groups of three charges parallel to each of the faces. This configuration has also been found by J. Edmundson (personal communication) and by Ashby and Brittin (*Am. J. Phys.* **54**, 776; 1986). Interested readers are referred to the unpublished work of Edmundson (May and Baker Ltd, Dagenham, Essex RM10 7XS, UK), who has made an almost exhaustive search of all possible configurations up to $N = 50$. Finally, the Föppl notation for the last entry in Table 1 ($N = 20$) contains a misprint and should read $13^2 63^2 1$.